COMPARISON OF PLEUROTUS OSTREATUS AND RHIZOPUS OLIGOSPORUS FUNGI FOR LOOSE SAND IMPROVEMENT

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ABSTRACT

Loose sand is a type of soil with a cohesionless particle or small soil cohesion value which results in low shear strength of the soil. This study attempts to identify an alternative for enhancing the shear strength of loose sand by employing the growth of the fungi *Pleurotus ostreatus* and *Rhizopus oligosporus* as binding agents. This option was done by combining the loose sand with Oyster spawn for the *Pleurotus ostreatus* soil sample and Tempeh inoculum for the *Rhizopus oligosporus* soil sample. Variations in water content and curing period were undertaken to investigate the influence of fungi development on improving the shear strength using the unconfined compression test. The results showed that the *Pleurotus ostreatus* soil sample produced a maximum unconfined compressive strength value of 73 kPa with 5 percent water content, 30 grams of Oyster spawn, and cured for 14 days whereas the *Rhizopus oligosporus* soil sample produced a maximum unconfined compressive strength using the unconfined source of Tempeh inoculum, and cured for 4 days. Moreover, a Scanning Electron Microscope test was also done to detect the variations in characteristics and the typical hyphae for both fungi.

Key words: Loose sand, soil improvement, Pleurotus ostreatus, Rhizopus oligosporus, bio geotechnics.

1. INTRODUCTION

Sand with poor grading and in a loose state has a medium to high surface erosion potential as well as a high wind erosion potential (Rivas 2006). Another technique for minimizing the potential is to increase the shear strength of loose sand. One of these methods is bio-mediated treatment, which consists of techniques that can improve a variety of soil properties such as permeability, stiffness, compressibility, shear strength, and volumetric behavior (DeJong *et al.* 2010). Microbial biotechnology, which is derived from biological sources such as bacteria, fungi, and algae, is a lowcarbon and environmentally friendly option. Over the last decade, it has evolved as a subdiscipline (Salifu *et al.* 2021).

Examples of treatment using bacteria are microbial-induced calcite precipitation (MICP) and enzyme-induced calcium carbonate precipitation (EICP). MICP has shown the ability to improve soil properties by cementing particles (DeJong *et al.* 2006; Teng *et al.* 2020; Gupta *et al.* 2021). It has also been shown to help minimize wind and water-induced erosion (Bang *et al.* 2011), as well as enhance liquefaction resistance (Montoya *et al.* 2013, Huang *et al.* 2021). EICP induces carbonate precipitation without the need for microorganisms by using plant-derived urease enzymes. The free enzyme is several orders of magnitude smaller than ureolytic microorganisms and is incapable of generating bio-films or extrapolymeric compounds, minimizing bioplugging, and broadening the range of application to finer-grained soils (Hamdan

2015). Differences between the two techniques in macro and micro scales have been reported by Nafisi *et al.* (2019). One challenge in these techniques is controlling the uniformity of cementation that occurred in the treated soil.

Salifu (2019) reported the influence of fungal growth on soil erodibility. In his experimental work, Pleurotus ostreatus was applied to the sand. In addition, laboratory jet erosion experiments were performed to establish the erodibility properties of the treated sands and control specimens. All specimens were made by combining soil (94 percent fine sand and 6 percent lignocel) with liquid (spores/hyphal suspension for treated or deionized water for untreated) to achieve a moisture content of 11.1 percent. There were 4 treatment methods namely fully-treated, half-treated, surface treated, and untreated. The untreated or control specimens were incubated for only 3 weeks meanwhile the rest of the specimens were incubated for 1, 3, 6, and 9 weeks. The study concluded that following a growth period of 3 weeks, fungal treated sands gain a significant increase in critical shear stress as well as a decrease in erodibility coefficient when compared to untreated sands. The time of growth also influences the resilience of fungal-treated sands, with a one-week growth period inadequate for enhancing erosion resistance. Furthermore, between the different treatment methods, there were no significant differences in the erosion susceptibility beyond a 3 weeks growth duration. Moreover, Lim et al. (2020) used the inoculum of Rhizopus oligosporus mixed with loose sand for their research. The dosage of the inoculum, the water content of the loose sand, as well as the curing time, were observed. It was found that an increase in dosage of inoculum produces a higher value of unconfined compressive strength (q_u) . A 5.24% dosage of inoculum, 5% water content, and 3 days of curing time yield the maximum q_u of 68 kPa.

Furthermore, the use of fungi for soil improvement is restricted to plant-mycorrhizal systems (Mardhiah *et al.* 2016). The goal of using mycorrhizal fungi is to promote plant growth to successfully revegetate degraded soil systems caused by soil erosion, landslides, or desertification (Requena *et al.* 2001; Caravaca *et al.*

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2003). It may aid in increasing root production, root length density, and, in some cases, plant root tensile strength (Stokes *et al.* 2009). Peng *et al.* (2013) discovered that the involvement of plant roots and hyphal networks has a positive impact on the stability of soil aggregates.

Based on the above literature, it has been proven that Rhizopus oligosporus and Pleurotus ostreatus could be used as a binding agent for loose sand and thus becomes a viable option for soil improvement. However limited study has been conducted to investigate this topic in detail. Both fungi are non-pathogenic, nonparasitic, and they are not harmful to environment. This paper focuses on the effects of curing time and water content on compressive soil strength using both Rhizopus oligosporus and Pleurotus ostreatus. Furthermore, the result of this study would be compared with the previous result (Lim et al. 2020). Some significant differences are the sand used in this study has a different soil gradation from the previous study. In addition, another fungus, namely Pleurotus ostreatus also used in this study to investigate the effectiveness of this fungus for increasing soil shear strength. Although Salifu (2019) has reported the loss in peak shear strength when Pleurotus ostreatus was introduced to the sand, it seems that it is still worth it to be checked using the way this paper prepared the soil sample.

2. METHODS

Silica sand was selected as the soil sample in this research. Before the sand was used, the sand was rinsed with tap water to clean the residue that might be found. Afterward, the sand was boiled for about 15 minutes at 100°C. After boiling, the sand was drained at room temperature for around 30 minutes, then it was put inside an oven for 24 hours and 105°C. This action was performed to replace the function of an autoclave which is usually used to sterilize the sand. After 24 hours, the sand was taken out from the oven, and cooling with room temperature before it is ready to be used. The room temperature is around 24°C to 28°C. The unfinished soil sample was placed inside a plastic container and sealed with tape to avoid contamination.

The index properties of silica loose sand are presented in Table 1. Figure 1 shows the gradation curve of silica sand based on the sieve analysis (ASTM-D6913). According to the Unified Soil Classification System (USCS), silica loose sand is categorized as poorly graded sand (SP). The dry unit weight of loose sand is 14.8 kN/m³

Table 1 Index properties of silica loose sand

Parameter	Value	
Water content (ω)	1.69%	
Specific gravity (G_s)	2.64	
Unit weight (γ)	15.1 kN/m ³	
Dry unit weight (γ_{dry})	14.8 kN/m ³	
Maximum dry unit weight ($\gamma_{dry-max}$)	17.8 kN/m ³	
Minimum dry unit weight ($\gamma_{dry-min}$)	14.5 kN/m ³	
Permeability (k)	0.024 cm/sec	
Internal friction angle at $D_r = 40\%$	30°	
Coefficient of uniformity (c_u)	2.25	
Coefficient of curvature (c_c)	1	
D_{10}	0.6 mm	
D_{30}	0.9 mm	
D_{60}	1.35 mm	
Soil classification	SP (poorly graded sand)	



Fig. 1 Gradation curve of silica sand

and its specific gravity is 2.64. The water content is about 1.69%, which is negligible. In this experiment, the sand was assumed completely dry for ease of weighting the fungi dosage for each series of experiments because the dosage is according to a percentage of the dry weight of the sand. The result of XRF is summarized in Table 2. As shown in Table 2, the main component was silicon dioxide (SiO₂), which is a component of quartz material.

As shown in Fig. 2, the fungi *Pleurotus ostreatus* (Oyster fungi inoculum) and *Rhizopus oligosporus* (Tempeh inoculum) were obtained from the commercial market. The Tempeh inoculum used is Raprima, which is manufactured in Indonesia, and the

Table 2 The result of XRF analysis

Percentage (%)
98.200
0.289
0.023
0.047
0.024
0.057
0.015
0.018
0.011
1.316



Fig. 2 The photos of (a) *Pleurotus ostreatus* spawn and (b) *Rhi-zopus oligosporus* inoculum

Oyster fungi inoculum is from a farmer's harvest purchased from a local store in Bandung, Indonesia. Because the Oyster fungi inoculum was grown on dry corn, it appears granular. Meanwhile, because it is mixed with rice flour, Tempeh inoculum resembles white powder.

2.1 Sample Preparation

The samples were made by combining silica sand with either Oyster spawn or Tempeh inoculum. The amount of silica sand used was kept at around 150 g. The soil mixture was then sprayed with distilled water until it was homogeneous. The mixed soil was then poured into the cylindrical mold. The relative density was about $40 \pm 2\%$. The mold's dimensions were based on the standard sample of an unconfined compression test (ASTM-D2166), which is 76 mm tall and 38 mm in diameter. Furthermore, the samples were kept at room temperature indoors. The temperature is around 24°C to 29°C. In addition, a perforated thin plastic sheet was place at the top and the bottom of the cylindrical mold to cover the soil sample and air could be circulated through the soil sample. Figure 3(a) shows the cylindrical mold and Fig. 3(b) shows the soil sample after preparation was done. The amounts of distilled water, oyster spawn, tempeh inoculum, and curing time vary depending on the experimental plans, as explained in the following section. Before testing, the samples were extruded with a laboratory sample extruder. The preparation of oyster spawn in this study differed from the preparation done by Salifu (2019).



Fig. 3 The photos of (a) cylindrical mold and (b) sample after casted into the mold

Salifu (2019) mixed the fungal inoculum with deionized water to create a fungal suspension. The soil sample was exposed to this fungal suspension. Until now, it appears that there is no standard method for preparing oyster spawn, and this different methodology is worth investigating further in the future.

2.2 Experimental Program

Table 3 summarizes the *Pleurotus ostreatus* experimental program. This study included two series of experiments with varying amounts of fungi inoculum, distilled water, and curing time. In the first experiment, samples were prepared with 5% water content, 30 g of Oyster spawn, and a curing time variation. The goal of Series 1 was to investigate the effect of curing time on q_u of mixed soil. Samples for Series 2 were prepared with a constant curing time and amount of Oyster spawn, 14 days and 30 g, respectively, and varying the water content. Series 2's goal is to examine the effect of water content on the unconfined compressive strength of treated sand.

Table 4 lists the *Rhizopus oligosporus* experimental program. Series A examined the performance and mechanism of fungi growth using Tempeh inoculum by varying the curing times of samples from 1 to 10 days. Furthermore, Series B performs the same function as Series 2 but for Tempeh inoculum. Finally, Series C and D were carried out to investigate the effects of sand gradation by comparing the results with those obtained by Lim *et al.* (2020).

3. **RESULTS**

3.1 Effects of Curing Time

Figure 4 depicts the growth of Oyster fungi during the curing time. It is clear that the Oyster fungi can cover the entire surface of the sand. As shown in Fig. 4, the samples with 14-day and 21-day curing times have denser fungi growth than the other curing days. The unit weight of treated sample with Oyster fungi is about 15.01 to 15.82 kN/m³. All treated samples were tested with the unconfined compression test to determine the unconfined compressive strength.

Table 3	List of ex	perimental	programs fo	or Oyster	fungi (<i>F</i>	Pleurotus ostreatus)
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Series	Water content (%)	Curing time (days)	Oyster spawn weight (g)	Objectives
1	5	3, 7, 14, 21, 28	30	To examine the influence of curing time on unconfined compressive strength
2	0, 5, 10, 15	14	30	To examine the influence of water content on unconfined com- pressive strength

Note: the water content is compared to the mass of dry silica sand

 Table 4
 List of experimental programs for Tempeh fungi (*Rhizopus oligosporus*)

Series	Water content (%)	Curing time (days)	Tempeh inoculum (%)	Objectives	
А	5	1, 2, 3, 4, 5, 6, 7, 8, 9, 10	5	To examine the influence of curing time on unconfined compressive strength	
В	0, 3, 5, 10, 15, 20	3	5	To examine the influence of water content on unconfined com- pressive strength	
С	0, 3, 5, 10, 15, 20	3	3.93	To examine the effects of sand gradation on unconfined compres-	
D	5	3	0.79, 1.31, 1.83, 2.62, 3.93, 5.24	sive strength	

Note: the percentage of water content and Tempeh inoculum is compared to the weight of dry silica sand



Fig. 4 The photos of Oyster fungi growth for (a) 3 days, (b) 7 days, (c) 14 days, (d) 21 days, and (e) 28 days

Figure 5 depicts the growth of Tempeh fungi during the scheduled curing time. From the first day (Fig. 5(a)), the fungi can see grew in the sand and the sample can stand alone. Starting from the 6-days, the fungi start to deteriorate, which can be seen from the fungi change to yellowish color. This deterioration keeping continues and the experiment stops in the curing time of 10-days. The unit weight of treated sample with Tempeh fungi is about 15.11 to 16.11 kN/m³.Similar to the Oyster fungi samples, these treated Tempeh fungi were tested using an unconfined compressive machine.

Figure 6 depicts the relationship between q_u and curing time of *Planteorus ostreatus* and *Rhizopus oligosporus*-treated samples.

It was discovered that the q_u of Oyster fungi steadily increased until 14 days of curing time. The q_u could reach 73 kPa at the optimum curing time of 14 days, which is equivalent to the q_u of medium clay soil (Terzaghi and Peck 1967). Furthermore, when the curing time exceeds 14 days, the q_u drops precipitously until it reaches 28 days. The q_u is approximately 7.3 kPa after 28 days. Tempeh fungi reached peak q_u on day 4 and then declined. The peak q_u is approximately 100 kPa, which is greater than the peak shear strength of the Oyster fungi-treated sample. Furthermore, it appears that Oyster fungi are more durable than Tempeh fungi. Oyster fungi may have existed for longer than fungi Tempeh. Tempeh fungi can survive for about 10 days, while Oyster fungi



Fig. 5 The photos of Tempeh fungi growth for (a) 1 day, (b) 3 days, (c) 5 days, (d) 6 days, (e) 8 days, and (f) 10 days





can survive for about 30 days. Although the strength of Oyster fungi is lower than that of Tempeh fungi, it should be noted that Oyster fungi may be more beneficial in practice due to their ability to withstand longer than Tempeh fungi.

Figure 7 depicts the comparison between the before and after compression test of the sample treated with Oyster fungi (14-day



Fig. 7 The comparison between before and after compression test of (a) Oyster fungi (14-day curing time), and (b) Tempeh fungi (5-day curing time)

curing time) and Tempeh fungi (5-day curing time). Those samples were selected as representative samples because they yield the maximum compressive strength during the scheduled curing time. After the compression test, a clear failure line occurred for the Oyster fungi sample. Meanwhile, for the Tempeh fungi sample, the failure line is not as clear as the Oyster fungi sample, and the shape of the sample was bulging.

The stress-strain curves of Oyster fungi and Tempeh fungi treated with sand with different curing times are shown in Fig. 8. The Tempeh fungi have a higher unconfined compressive strength than the Oyster fungi after three days of curing. However, the trend was reversed for a 7-day curing time. The strain-hardening behavior is visible in the stress-strain curves. Furthermore, Young's modulus (E) of all samples is approximately 1,300 kPa.

3.2 Effects of Water Content Sample Treated with *Pleurotus Ostreatus*

Figures 9 and 10 show photos of Oyster fungi growth and Tempeh fungi growth with varying water content. According to visual inspection, the higher water content resulted in poor growth of Oyster fungi. This was not the case with Tempeh fungi. The appearance of Oyster fungi mycelium is more likely to resemble a white curtain adhering to soil particles, whereas the appearance of Tempeh fungi mycelium is more likely to resemble kinds of cotton binding to soil particles.

Figure 11 depicts a comparison of the unconfined compressive strength of Oyster fungi and Tempeh fungi at various water content levels. The ideal water content for both Oyster and Tempeh fungi is 5%. The q_u tends to decrease when the water content is greater than 5%. After three days of curing, the q_u of Oyster fungi and Tempeh fungi is very similar, with a water content of less than 5%. However, as shown in Fig. 6, this trend may not be the same for different curing times. The maximum q_u of Tempeh fungi and Oyster fungi at 5% water content is 95 kPa and 72 kPa, respectively.



Fig. 8 The stress-strain curves of Oyster fungi and Tempeh fungi treated sand with different curing times



Fig. 9 The photos of Oyster fungi growth with water contents of (a) 5%, (b) 10%, and (c) 15%



Fig. 10 The photos of Tempeh fungi growth with water contents of (a) 5%, (b) 10%, and (c) 15%



Fig. 11 The comparison between unconfined compressive strength of Oyster fungi and Tempeh fungi with various water contents

Figure 12 depicts the stress-strain curve of treated soil with varying water content. According to the findings, Tempeh fungi had a higher unconfined compression strength than Oyster fungi. It should be noted that in this series, the curing time was three days, with Tempeh fungi yielding greater strength than Oyster fungi. Furthermore, when the water content is 15%, there is a clear gap in Young's modulus of Tempeh fungi and Oyster fungi treated sand. Tempeh fungi have a higher Young's modulus than Oyster fungi. Tempeh fungi have Young's modulus of 1,160 kPa and Oyster fungi have Young's modulus of 286 kPa when the water content is 15%.

3.3 Effects of sand Gradation

Series C and D were carried out to compare with the data obtained from Lim *et al.* (2020). Lim *et al.* (2020) used Padang sand as the soil sample in this paper, whereas silica sand was used in this paper. The gradation curve, as shown in Fig. 13, distinguishes



Fig. 12 The stress-strain curves of Oyster fungi and Tempeh fungi treated sand with water contents of (a) 5%, (b) 10%, and (c) 15%



Fig. 13 Grain distribution of silica sand and Padang sand

these sands. Padang sand has a balanced proportion of fine and coarse to medium particles, whereas silica sand is dominated by coarse to medium sand and is classified as poorly-graded sand (SP). In this study, the comparison was limited to Tempeh fungi.

The graphs in Fig. 14 show the relationship between unconfined compressive strength and water content, as well as the relationship between unconfined compressive strength and Tempeh inoculum dosage. According to Fig. 14, Padang sand appears to have a better performance because it yielded a higher q_u value. Furthermore, Tempeh fungi can grow in Padang sand with higher water content. For the tempeh dosage, it appears that the results were quite similar between Padang sand and silica sand, though Padang sand had a slightly higher q_u . Based on these findings, it is possible to conclude that Tempeh fungi grew better in fine sand.

4. MICROSTRUCTURE OF SOIL TREATED WITH FUNGI

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The microstructure of mycelium bonds among sand particles was examined using a scanning electron microscope (SEM). This experiment used four samples, two from each fungus. The first sample (Fig. 15(a)) has an 8-day growing period, while the second sample (Fig. 15(b)) has a 28-day growing period. The third Tempeh inoculum sample (Fig. 15(c)) has a 2.62 percent Tempeh inoculum dosage with a 5-day growing period. The fourth sample (Fig. 15(d)) has a 5% Tempeh inoculum dosage and a growing period of 42 days. The sand particles can be seen bonding with the mycelium of their respective fungi in Figs. 15(a) and 15(b), though the way they bond is noticeably different. Pleurotus ostreatus bonded the particles by enveloping them, whereas Rhizopus oligosporus connects the particles with web-like threads, as shown in Figs. 15(c) and 15(d). It is also clear that the hyphae are still bonding to the soil particles although several days have passed. The q_u of these samples is very small, according to the unconfined compression test (almost zero). It denotes the hyphae strength that contributes to the unconfined compressive strength of the treated samples. The strength of the hyphae would decrease as it weathered. However, it can still keep the sample's cylindrical shape.

Rhizopus oligosporus and *Pleurotus ostreatus* hyphae are depicted in Fig. 16. According to Fig. 16, the Tempe hyphae is thicker than the Oyster hyphae. This discovery might explain why Tempeh fungi-treated samples had higher unconfined compressive strength than Oyster fungi-treated samples. The thicker the hyphae, the stronger the hyphae's strength. Furthermore, the fungi coverage area of Oyster fungi appears to be larger than that of Tempeh fungi. This could explain why the Oyster fungi are more durable than the Tempeh fungi (Fig. 6).



Fig. 14 (a) The relationship between unconfined compressive strength and water contents (b) the relationship between unconfined compressive strength and Tempeh inoculum dosage



Fig. 15 SEM images of treated samples: (a) Oyster fungi: 8 days, (b) Oyster fungi: 28 days, (c) Tempeh fungi: 5 days, and (d) Tempeh fungi: 42 days



(a)

(b)

Fig. 16 SEM images magnified at 600 times of (a) Oyster fungi hyphae and (b) Tempeh fungi hyphae

5. DISCUSSIONS

According to the findings, the main challenge of using fungi for soil improvement appears to be durability. One of the known reasons is due to the treated sample's lack of nutrients for the growth of fungi. Carbohydrates (such as starch) and calcium compounds (such as gypsum) are added to mycelium composites as nutrients for fungus and pH adjusters for the medium, respectively (Kuribayashi *et al.* 2022; Holt *et al.* 2012; Islam *et al.* 2018). According to Ongpeng *et al.* (2020), the rice barn, sawdust, and coconut husk contain nutrients that achieve mycelium growth matrix. Those three materials are categorized as lignocellulosic substrates. Moreover, according to Elsacker *et al.* (2019), the main factors affecting the production of mycelium composites, and consequently their mechanical behavior, are the matrix (mycelium species), the feedstock selection (lignocellulosic substrate), the interaction between white rot fungi and their feedstock and last but not least the process variables during manufacturing (protocol, sterilization, inoculation, packing, incubation, growing period and drying method). Studies have demonstrated that the mechanical properties of mycelium composites are mostly affected by their feedstock (Ziegler *et al.* 2016; Haneef *et al.* 2017; Appels *et al.* 2018). Hence, adding lignocellulosic substrates, carbohydrates, and calcium compounds to the soil-fungi mixing composition is worth to be conducted in future investigations.

Furthermore, Salifu (2019) demonstrated that Oyster fungi have a good performance to reduce the erodibility coefficient of soil during 3 weeks growth period. Moreover, the simple molded mycelium composites are often ideal for fill materials due to their porosity and lightweight rather than load-bearing applications (Kuribayashi *et al.* 2022). The fungi bricks also have been successfully developed and have greater compressive strength than the non-fungi bricks (Ongpeng *et al.* 2020). According to the experimental finding, the fungi only can live in humid/moist soil conditions. Hence, it could be said that it could not be used for sand under groundwater level. Even though the use of fungi in geotechnical engineering has yet to be thoroughly investigated, it appears to be a promising alternative for soil improvement.

6. CONCLUSIONS AND RECOMMENDATIONS

In this study, laboratory tests were conducted to compare some soil properties of sand treated with Tempeh fungi and Oyster fungi. Several conclusions and recommendations may be drawn:

- 1. The unconfined compressive strength of Tempeh fungi treated sand is greater than that of Oyster fungi treated sand for a short period (such as 4 days). However, after 6 days, the strength of Tempeh fungi treated sand had significantly decreased. Furthermore, Oyster fungi treated sand lasts longer than Tempeh fungi treated sand.
- 2. The ideal water content for both Oyster and Tempeh fungi is 5%. The q_u tends to decrease when the water content is greater than 5%. In addition, Tempeh fungi flourished better in Fine sand.
- 3. The *Pleurotus ostreatus* bonded the particles by enveloping them, while the *Rhizopus oligosporus* connects the particles with web-like threads, according to SEM. Furthermore, the fungi coverage area of Oyster fungi appears to be larger than that of Tempeh fungi. This could explain why the Oyster fungi are more durable than the Oyster fungi.
- 4. Adding lignocellulosic substrates, carbohydrates and calcium compounds to the soil-fungi mixing composition are worth to be conducted in the future investigation to increase the durability and the growth of mycelium.

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DATA AVAILABILITY STATEMENT

Some or all data, models, or code that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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