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Insights into the impact of synergistic fungi-biopolymer stabilization on the shear strength of sand

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ABSTRACT

In the past decade, there have been several studies on the geotechnical strength of biopolymer-treated soils. These studies indicated that a major disadvantage of biopolymer treatment is its inability to maintain adequate strength upon soil saturation. To address this drawback, a new stabilization technique has recently been explored whereby biopolymer addition is combined with the inoculation of fungi. The synergic application of fungi and biopolymers to soil stabilization is a novel and promising approach but it remains to be fully validated. In this study, an experimental campaign was carried out to identify the best nutrient media among sugar, honey and Guar gum while exploring the best fungi-biopolymer combination for soil stabilization. The shear strength of a treated sand was investigated by direct shear tests at different stages of fungal growth. Test results indicated that sugar is the best nutrient due to a simple structure that is easily broken down by fungi. Moreover, the combination of 5% fungal liquid suspension (obtained from 2% solid fungal spawn and 3% sugar) and 1.5% biopolymer (Xanthan gum) appeared optimal for enhancing soil cohesion.

INTRODUCTION

The increasing demand for earthen infrastructure has led to the depletion of suitable soil deposits, underscoring the need for ground stabilization. The construction industry has mainly relied on chemical stabilization, particularly by cement additives, to improve the mechanical properties of soils. However, cement production contributes significantly to global carbon emissions, accounting for up to 8% (Supriya et al. 2024, Belaid 2022). Cement stabilization may also raise the soil pH to harmful levels up to 9 (Taylor 1997, Hansen 2002), disrupting soil ecology and

irreversibly altering soil properties, which in turn may increase pollution of surface runoff and groundwater (Msimbira and Smith 2020). These environmental impacts highlight the urgent necessity of sustainable alternatives to cement for soil stabilization.

Amongst the different sustainable soil stabilisation techniques studied in recent years, bio-based methods have emerged as a promising innovation (DeJong et al. 2017, Salifu and Mountassir 2021). Fungi-based methods have recently surfaced as a viable option among bio-stabilization techniques. Fungi have been extensively researched and applied to different technological fields such as, for example, the enhancement of crop growth (Athinuwat et al. 2024), the remediation of heavy metals in contaminated soils (Babu et al. 2014) and the control of wind erosion (Tisdall et al. 2012). A handful of studies have also focused on the use of fungi to stabilize soils by improving their mechanical properties. El Mountassir et al. (2018), recently made a case for exploring soil stabilization by the hyphal/mycelium network of filamentous fungi. Filamentous fungi grow in long thread-like structures, i.e. the hyphae, which form an interconnected three-dimensional mesh known as the mycelium. These filamentous fungal species grow near the soil surface, forming an extensive network of hyphae that can stretch several metres (Salifu and Mountassir 2019). Fungi enhance soil aggregation (Baumert et al. 2018), which is a crucial requisite to increase material strength (Lim et al. 2020). Pioneering studies showed that fungal-treated soils exhibit greater strength and water-repellence, even at small fungal dosages, i.e. 1-5% relative to soil mass (Salifu 2019). Therefore, fungi hold massive potential as an alternative to cement for preventing instabilities and erosion in slopes and embankments. The growth of filamentous fungi is heavily dependent on the organic matter used as a nutrient, which must aid mycelium growth without hindering soil stabilisation. Among the organic substrates promoting fungal growth, biopolymers stand out as a promising choice. The use of biopolymers is advantageous not only for their ability to serve as a nutrient for mycelium growth but also for their inherent strengthening properties. Biopolymers serve a compelling dual function: they promote the development of fungal networks while simultaneously enhancing soil strength. The strengthening effects of biopolymer-treated soils have been observed to persist for up to two years (Chang et al. 2015), making them an ideal choice for sustainable stabilization.

A major concern with using biopolymers alone is the inability of the ‘hydrogels’ formed by biopolymers to resist dissolution on water ingress. This drawback, however, could potentially be offset by the mycelium which increases the water-repellence of soils (Park et al. 2023). In this respect, optimally dosed biopolymers can provide a vital nutrient source for fungal growth while also enhancing soil strength. The synergy of fungi and biopolymer will not only promote fungal proliferation and soil stabilisation but will also contribute to the long-term soil durability, thus presenting an attractive alternative to conventional stabilization methods. The main objective of this study is to identify the optimal fungal-biopolymer combinations and nutrient conditions that promote effective mycelium growth. Different fungal liquid suspensions were prepared from a solid spawn of *Pleurotus Eryngii* fungus by using various nutrients (honey, Guar gum, sugar) in different proportions. The liquid suspensions were added to a sandy soil along with the biopolymer Xanthan gum in varying combinations to identify the optimum dosages. A series of direct shear tests were finally performed on the treated soils to quantify the level of strength improvement.

MATERIALS

Soil: Leighton Buzzard sand, which is a well-graded fine silica sand of high quality and uniformity, was used as the base soil in this study. The particle size distribution was measured following BS

1377-2:1990 (1990), which confirmed the uniform grading of the sand. This soil was first washed with tap water on a 600μm sieve to remove any contaminants, soluble salts, and finer particles. After that, it was rinsed with distilled water to ensure consistency and purity, thus preventing potential chemical reactions. The soil was finally oven-dried for 24 hours at 105°C before use.

Fungi: Solid spawn of *Pleurotus Eryngii* fungi species was used as the mycelium source in this study. Solid spawn is a fungal mycelium which is grown on a substrate material (like grains, sawdust, straw, or wood chips) previously inoculated to propagate the fungus into new environments. The solid spawn was supplied from M/s Merit Mushrooms, UK, as sterilized and hydrated wheat grains previously inoculated with a pure mycelium culture. As recommended by the supplier, the spawn was stored in a refrigerator at a temperature between 2 and 5°C.

Biopolymer: Xanthan gum and Guar gum biopolymers were supplied in powdered form by Merck under the M/s Sigma-Aldrich brand. The supplier recommends storing the biopolymers in airtight lids, in a cool and dry place with clear labelling and away from potential contaminants. Xanthan gum has been shown to have better strength improvement properties when used for soil stabilization compared to Guar gum (Muguda et al. 2020).

EXPERIMENTAL PROCEDURE

Fungi inside the solid spawn are in the final stage of growth and are supplied along with their nutritional source integral to the spawn. Preliminary tests by the authors revealed that the direct introduction of solid spawn inside the soil does improve strength. Nevertheless, the presence of leftover nutritional sources, like wheat brawn, can also create weak material spots and, in this study, it was therefore preferred to employ fungi in liquid suspensions which are free from such impurities. A series of preliminary tests were conducted to prepare liquid fungal suspensions from the solid spawn using different nutritional media to optimize growth within the soil.

a) Inoculation of liquid suspension from solid spawn

Liquid fungal suspensions were produced from the solid spawn using three nutrient sources: honey, sugar, and Guar gum. Conical flasks of 500 ml capacity were filled with 200 ml of deionized water, to which various combinations of nutrient sources and solid spawn were added as listed in Table 1 (the percentages in the table have been calculated with respect to distilled water). Even small differences in fungal spawn dosage can significantly impact the growth rate and spatial extent of hyphal development, particularly in this study where growth is observed under controlled conditions such as petri dishes and for a limited 21-day period. The solid spawn was initially transferred into the flasks containing the water with the corresponding nutrient source. The flasks were then vigorously shaken (Fig. 1a) for 15 minutes to separate the mycelium from the spawn and the content was then allowed to settle for 5 minutes before being again vigorously shaken for another 10 minutes. The samples were subsequently incubated in a climatic chamber at a temperature of 25°C and relative humidity of 50% to allow the growth of hyphae inside the suspension. The liquid suspension was then carefully withdrawn using a pipette leaving any solid matter inside the flask (Fig. 1b).

Table 1. Constituents of fungal liquid suspensions (LS)

	Fungi solid spawn	Honey	Sugar	Guar gum

Fungi LS 1	2%	3%		
Fungi LS 2	2%		3%	
Fungi LS 3	2%			0.075%
Fungi LS 4	2.50%	3%		
Fungi LS 5	2.50%		2%	
Fungi LS 6	2.50%			0.20%

b) Preparation of soil sample

The soil and the Xanthan gum biopolymer were first dry mixed to achieve a target skeletal dry density of 1.4 g/cm³ (Fig. 1c). Such a low density was adopted to ensure that the fungal mycelia would get enough pore spaces to develop within the soil (Gou et al. 2021). The dry mix was then amended with the fungal liquid suspension (Fig. 1d) at varying dosages of 3% and 5%. For the 3% dosage, distilled water was also added to ensure homogeneity, while maintaining a fixed total moisture content of 5%. The amended soil was transferred into a 90 mm diameter petri dish. Liquid suspensions were introduced cultured for 1, 2 and 3 days since preparation to monitor the effect of fungal growth on mycelia production. The dosage of the biopolymer Xanthan gum was also varied as either 1.5% or 2.5% of the dry soil mass.

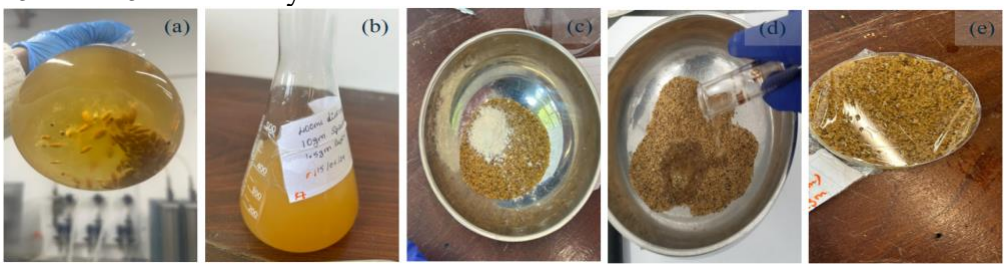

Figure. 1. Sample preparation: (a) deionised water, nutrient source and solid spawn being shaken to separate mycelium from spawn (b) separated liquid fungal suspension (c) dry mixing of xanthan biopolymer and soil (d) adding fungal liquid suspension to soil-biopolymer mixture (e) amended soil specimens in petri dish covered by cling film

Table 2 provides a summary of the petri dish soil samples corresponding to different combinations of fungal suspension and Xanthan gum dosages. As mentioned, each combination was tested with fungal liquid suspensions cultured for 1, 2, and 3 days after preparation, resulting in a total of 72 samples. The petri dishes were covered with cling film punctured with 25 holes (Fig. 1e) to prevent excessive moisture loss while ensuring an adequate supply of oxygen for fungal growth (Bettin 2020). They were then incubated inside a controlled environment at a temperature of 25°C and a relative humidity of 50% according to suggestions by both Salifu et al. (2024) and the fungi supplier. Salifu et al. (2024) found that these environmental conditions were optimal for the growth of *Pleurotus Ostreatus*, which belongs to the same family and species as *Pleurotus Eryngii*.

Table 2. Fungi-biopolymer combinations of treated soil samples

Combination No.	Fungal suspension	Xanthan gum	Distilled water	Combination No.	Fungal suspension	Xanthan gum	Distilled water
1	LS1 3%	1.5%	2%	13	LS4 3%	1.5%	2%
2	LS2 3%	1.5%	2%	14	LS5 3%	1.5%	2%
3	LS3 3%	1.5%	2%	15	LS6 3%	1.5%	2%
4	LS1 3%	2.50%	2%	16	LS4 3%	2.50%	2%

5	LS2 3%	2.50%	2%	17	LS5 3%	2.50%	2%
6	LS3 3%	2.50%	2%	18	LS6 3%	2.50%	2%
7	LS1 5%	1.5%	0%	19	LS4 5%	1.5%	0%
8	LS2 5%	1.5%	0%	20	LS5 5%	1.5%	0%
9	LS3 5%	1.5%	0%	21	LS6 5%	1.5%	0%
10	LS1 5%	2.50%	0%	22	LS4 5%	2.50%	0%
11	LS2 5%	2.50%	0%	23	LS5 5%	2.50%	0%
12	LS3 5%	2.50%	0%	24	LS6 5%	2.50%	0%

(c) Direct shear tests

To explore strength improvement, direct shear tests were conducted on both treated and untreated soil samples. Samples were treated with selected fungi-biopolymer combinations based on the results of the previous optimisation study in petri dishes. The tests were performed in accordance with BS-1377-7 (1990) on samples of 60 mm x 60mm x 25 mm size under 3 different normal stresses of 25 kPa, 50 kPa and 100 kPa at a displacement rate of 1 mm/min.

Direct shear tests on untreated soil: The minimum dry density at which untreated soil samples could be prepared was 1.52 g/cm^3. Consequently, the shear strength of the untreated samples was measured at dry densities of 1.52, 1.58 and 1.65g/cm^3 and the strength at 1.4 g/cm^3 (i.e. the same dry density of treated samples) was estimated by linear extrapolation.

Direct shear tests on fungi-biopolymer treated soil: The treated soil, incorporating the fungal liquid suspension and the Xanthan gum biopolymer, was transferred inside the direct shear test mould in 3 equal layers. Each layer was lightly pressed 25 times using a rectangular wooden tamper to achieve a density of 1.4gm/cm^3. The samples were then extruded from the mould and wrapped with aluminum foil punctured with 25 holes on all sides to prevent excessive moisture loss while ensuring adequate oxygen supply for fungal growth. The samples were then carefully transferred inside a climatic chamber where they were maintained at a temperature of 25°C and a relative humidity of 50% to allow mycelium growth. Specimens were prepared in triplicates and were sheared after either 7 or 14 days since the start of incubation.

RESULTS AND DISCUSSION

The optimisation tests in petri dishes were conducted to identify the most effective nutrient source for the production of liquid suspensions from the solid spawn of *Pleurotus Eryngii* fungi. These tests also determined the optimal time of culture before introducing the suspension into the soil. Results revealed interesting mycelium growth patterns across different nutrient sources and culture times. Based on these findings, two combinations of liquid suspension and Xanthan gum biopolymer dosages were selected for further investigation inside the soil.

(a) Petri dishes tests

The fungal liquid suspension with sugar as a nutrient source consistently outperformed those made with honey and Guar gum, particularly after three days of culturing. The hierarchical performance of sugar, honey, and Guar gum as nutrient sources likely reflected their varying complexities and bioavailability. Even though Guar gum has a simpler composition, made of mannose and galactose units linked in a linear chain (Sharma et al. 2018), the fungi seem to prefer easily digestible and readily metabolizable carbon sources like sugar or honey. Guar gum, is a more complex galactomannan polysaccharide, requiring fungi to produce special enzymes to break it down. Between sugar and honey, the relatively simple chemical structure of sugar, consisting of

sucrose molecules, provides an easily accessible energy substrate for fungi and ensures rapid mycelial proliferation (Moore et al. 2011). Samples were assessed by visually comparing mycelium growth on days 3, 7 and 14.

Combination 8, consisting of 5% LS2 (2% solid spawn and 3% sugar) and 1.5% Xanthan gum (see Table 2), was selected for shear tests, as it produced the most visible, uniform and dense mycelium growth after 14 days (Fig. 2). Combination 23, consisting of 5% LS5 (2.5% solid spawn and 2% sugar) and 2.5% Xanthan gum (see Table 2), was also chosen, despite exhibiting less mycelial growth compared to Combination 8. The choice of Combination 23 aimed to assess potential variations in soil strength arising from differences in nutrient content and solid spawn dosage. It was noted that the percentage of nutrient media played a more significant role in promoting mycelia growth than the actual amount of solid spawn. Specifically, the liquid suspension LS2 from Combination 8 exhibited superior mycelia growth, while the smaller quantity of nutrient media in LS5 of Combination 23 may have been depleted within the initial 3-day period.

It was interesting to note that the samples amended with 1.5% biopolymer exhibited a better growth compared to those with 2.5% biopolymer. This suggests that an optimal biopolymer concentration exists for promoting fungal proliferation, beyond which growth is inhibited. Excessive biopolymer concentration might have created an overly viscous environment, potentially impeding hyphal extension and nutrient diffusion. This aligns with findings by Harris et al. (2006), who observed that soil physical properties, including viscosity, significantly influence fungal growth dynamics. Also, Salifu (2019) noted that biopolymer formed a sticky hydrogel cohering soil particles together and holding moisture in the soil, making it challenging for the mycelium to proliferate. Moreover, in samples amended with higher biopolymer content, clustered and localised growth was observed. It is believed that abundant nutrition source reduces the need for extensive foraging. Instead of hyphae spreading out to gather nutrients, the growth concentrates in nutrient-rich areas, which might explain the denser, localized mycelial growth as experimentally observed.

Figure. 2. Fungi mycelium growing in soil

(b) Direct shear tests

Direct shear tests were performed on soil specimens, treated with Combination 8 and Combination 23, after 7 and 14 days of incubation. The cohesion (c) and the angle of internal friction (ϕ) were determined from the peak strength measured at normal stresses of 25 kPa, 50 kPa and 100 kPa. Table 3 shows the cohesion and friction angle measured from all tests on treated and untreated soil samples. For the untreated soil, the value of cohesion was zero, while the friction angle was determined to be 30º at a dry density of 1.4 g/cm^3 by extrapolating measurements at higher densities, as explained earlier.

Test results revealed a significant increase in both cohesion and friction angle because of fungi-biopolymer treatment. Combination 8 showed a marked development of cohesion, reaching

117 kPa, and a rise in friction angle, increasing to 41°, after 7 days of treatment. This represents a 36.67% increase in friction angle compared to the untreated soil at the same density of 1.4 g/cm^3, and a 5.12% increase compared to the untreated soil at a higher density of 1.65 g/cm^3. The increase in friction angle might be attributed to the formation of a biopolymer hydrogel, which promotes binding between soil particles, as well as the growth of a fungal mycelium reinforcing the soil structure. By day 14, cohesion slightly increased to 118 kPa, while the friction angle decreased slightly to 38°. The additional binding by the biopolymer hydrogel, along with the continued particle enmeshment due to mycelial growth, likely contributed to further aggregation and bond strengthening, thereby enhancing cohesion. The decrease in friction angle between 7 and 14 days may instead be explained by increased fungal growth, with hyphae and fungal exudates (hydrophobin) coating soil grains, potentially acting as a lubricant between particles.

Combination 23 produced lower soil strength than Combination 8, despite both having the same liquid suspension dosage. Combination 23 was formulated with liquid suspension LS5, which contains a lower nutrient content compared to the liquid suspension LS2 used in Combination 8. This reduced nutrient level may have inhibited mycelial growth, leading to poorer aggregation of soil particles. Furthermore, the higher biopolymer dosage in Combination 23 could have limited the space available for hyphal growth and hindered fungal mycelial proliferation, contributing to the lower strength. This is further supported by the non-uniform clustered growth of fungal mycelium observed in the petri dish tests.

Finally, the shear strength parameters of the samples treated with Combination 23 exhibited an opposing trend of variation with culturing time, compared to those treated with Combination 8, as cohesion decreased while the friction angle increased from 7 to 14 days. The increase in friction angle may be attributed to the stiffening of the relatively large amount of biopolymer within soil pores, enhancing interlocking between particles and, consequently, leading to higher friction. Conversely, the decrease in cohesion may result from the reduced proliferation of fungal mycelium due to the stiffening of the excess biopolymer hydrogel over time, thus leading to limited mycelial bonding between particles.

Table 3. Shear strength parameters of untreated and treated soil samples

Specimen details	Days	c (kPa)	ϕ (deg.)
Untreated dry sand: 1.52 g/cm^3	-	0	34
Untreated dry sand: 1.58 g/cm^3	-	0	36
Untreated dry sand: 1.65 g/cm^3	-	0	39
Untreated dry sand: 1.4 g/cm^3	-	0	30
Treated by Combination 23 (LS5 5%, BP 2.5%)	7	72	32
	14	62	35
Treated by Combination 8 (LS2 5%, BP 1.5%)	7	117	41
	14	118	38

CONCLUSIONS

This study provides initial insights into the synergistic stabilization of a sandy soil using fungi and biopolymer. Specifically, it identifies a suitable nutrition source for culturing fungal liquid suspensions from solid spawn, as well as an effective fungi-biopolymer combination to enhance

soil shear strength. Sugar was found to be a superior nutritional source for *Pleurotus Eryngii* fungi, compared to honey or Guar gum, as the mycelial growth was visually more uniform and denser.

To characterize the shear strength of the stabilized soil, distinct combinations of sugar-based liquid suspensions and biopolymer (Xanthan gum) concentrations were tested. An optimal combination was identified, resulting in a significant enhancement of soil strength. The notable increases in both cohesion and friction angle are attributed to the physical enmeshment of soil particles by the mycelial network, as well as the adhesion induced by the biopolymer hydrogel within the soil.

While this research does not address the hydraulic characteristics of the treated soil, or the mechanical behavior under wet conditions, it successfully establishes the optimal conditions for fungal mycelium proliferation in the presence of biopolymer. This lays a foundation for future studies to explore critical aspects of treated soils such as the impact of fluctuating moisture environments on the hydro-mechanical behavior and long-term durability of the stabilized material. Furthermore, experiments on soils treated with biopolymer alone need to be performed to allow for a clearer understanding of the relative contribution to cohesion increase. A deeper study of hydraulic conductivity, erosion resistance and biopolymers degradation in presence of water will be crucial for fully realizing the potential of this innovative soil stabilization technique in engineering practice.

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