

## **Exploratory Research on Optimizing the Synergistic Application of Fungi Biopolymers in Soil Engineering**

**Alice Lane,<sup>1</sup> Sravan Muguda, Ph.D.,<sup>2</sup> Emmanuel Salifu, Ph.D.,<sup>3</sup> Hamed Khodadadi Tirkolaei, Ph.D.,<sup>4</sup> Agostino Walter Bruno, Ph.D.,<sup>5</sup> and Domenico Gallipoli, Ph.D.<sup>6</sup>**

<sup>1</sup>Department of Engineering, Durham University, Durham, DH1 3LE; E-mail: amqlane@yahoo.co.uk

<sup>2</sup>Department of Engineering, Durham University, Durham, DH1 3LE; E-mail: sravan.muguda-viswanath@durham.ac.uk

<sup>3</sup>Center for Bio-mediated and Bio-inspired Geotechnics, Arizona State University, Tempe, 85287-3005; E-mail: emmanuel.salifu@asu.edu

<sup>4</sup>Center for Bio-mediated and Bio-inspired Geotechnics, Arizona State University, Tempe, 85287-3005; E-mail: hkhodada@asu.edu

<sup>5</sup> Department of Civil, Chemical and Environmental Engineering, Università di Genova, Genova, 16145; E-mail: agostinowalter.bruno@unige.it

<sup>6</sup> Department of Civil, Chemical and Environmental Engineering, Università di Genova, Genova, 16145; E-mail: domenico.gallipoli@unige.it

### **ABSTRACT**

Human activities have accelerated soil degradation, highlighting the need for effective stabilising techniques. Conventional stabilisation practices involve energy and carbon intensive processes. Alternatively, significant soil stabilisation can be achieved with small amounts of biopolymer, while the saprotrophic fungus has been found to induce water repellency and stabilisation in sand. This exploratory study leverages the synergistic interaction between fungal networks of *Pleurotus eryngii* and biopolymers, specifically guar and xanthan gums. Three methods of fungal inoculation were assessed— solid spawn, liquid suspension and blend. The solid spawn showed the most promising fungal growth on a sand biopolymer composite, with recommendations provided for potential adjustments to future liquid inoculates. Furthermore, the impact of fungal growth over 14 and 21 days on the compressive strength of the biopolymer-sand composites was examined. Xanthan gum exhibits superior mechanical properties, while guar gum showed greater potential as a nutrient source.

### **INTRODUCTION**

Ground improvement techniques are employed to achieve at least one of the following objectives: an increase in bearing capacity, control of deformations, acceleration of consolidation, and lateral stability support. The most common methods are based on the fundamental principles of consolidation, densification, reinforcement, chemical stabilisation, thermal stabilisation, and load reduction (Patel 2019). Employing any of these principles requires energy in production and application, and the use of heavy machinery which releases greenhouse gases directly into the atmosphere. Furthermore, chemicals such as Portland cement extensively utilized for ground improvement, are hazardous or toxic to the soil and groundwater. Since 2018, the buildings and

construction sector has diverged from the goals of the Paris Agreement (United Nations Environment Programme 2022). Therefore, there is a need to develop more sustainable ground improvement techniques and/or to refine existing methods for the industry to get back on track. Over the last two decades, researchers have begun to explore the role of biological components in soils and their capacity for ground improvement. This has led to the emergence of a novel subdiscipline known as ‘bio-geotechnics’, which focuses on using biological process or products for soil stabilisation (DeJong et al. 2017).

Biopolymers such as Xanthan gum and guar gum have emerged as an alternative to traditional soil additives. Guar gum is derived from the guar bean, while xanthan gum is produced via fermentation of sugars using the bacteria *Xanthomonas campestris*. Both biopolymers have wide applications in the food, pharmaceutical, and other industries, therefore, are readily available at a competitive price (Bhat et al. 2022; García-Ochoa et al. 2000; Mudgil et al. 2014). The application of xanthan gum and guar gum in soil stabilization has been shown to increase cohesion, resulting in an improvement in bearing capacity, shear resistance and durability (Chang et al. 2015; Muguda et al. 2017; Qureshi et al. 2017; Soldo et al. 2020). In this respect, it has been reported that xanthan gum tends to exhibit superior performance compared to guar gum (Muguda et al. 2017, 2020). The main drawback to soil treatment with biopolymers is their hydrophilicity. Upon exposure to water, biopolymers transform into a hydrogel. In sandy soils, the hydrogel enhances cohesion but simultaneously diminishes surface dilatancy of the particles, resulting in a reduction of the friction angle (Lee et al. 2019). Another consideration is the time-dependent degradation of biopolymers. While this characteristic allows for environmentally safe disposal, it also limits the long-term stability and application of biopolymers in geotechnical projects (Chang et al. 2016). Managing the temporal degradation of biopolymers is essential for maintaining their sustainability and effectiveness over extended periods.

The use of vegetation growth to improve soil erosion has been recognized in engineering practices since the thirteenth century and numerous studies have investigated the associated geotechnical mechanisms (Cazzuffi et al. 2014). Root reinforcement has been found to improve shallow slope stability through mechanical and hydrological processes (Cazzuffi et al. 2014). Stabilisation by plant roots has been further studied in synergy with Arbuscular Mycorrhizal Fungi (AMF). A distinctive attribute of this group of fungi is the mutualistic relationship it forms with the plant roots. This symbiosis improves the properties of the surrounding soil and therefore provides advantageous geotechnical performance. The network of hyphae radiating from the root is referred to as mycelium and it binds the soil particles forming aggregates which stabilise the soil structure (Allen 2022). AMF have also demonstrated clear benefits in sustainable and organic agricultural systems, serving as a bio-fertilizer to replace chemical fertilizers (Allen 2022). However, AMF are biotrophic organisms, indicating their dependence on a host plant for survival. This limitation significantly restricts engineering applications as the fungi cannot be utilized in areas where host plants are absent or undesirable. Non-mycorrhizal (saprotrophic) filamentous fungi do not form symbiotic relationships with plants, and do not need a host plant to survive (Watkinson et al. 2015). They also produce mycelium, which contributes to soil aggregation. These fungi play a crucial role in nutrient cycling and organic matter decomposition in ecosystems (Watkinson et al. 2015). *Pleurotus ostreatus*, a saprotrophic, non-parasitic fungus, whose reproductive structure (mushroom) is the second most cultivated globally, has undergone initial screening for potential application in ground improvement engineering by Salifu (2019). Treatment of sand with *Pleurotus ostreatus*, using lignocellulose (plant fiber) as the source of organic matter, was found to create a water repellent barrier (Salifu and El Mountassir 2021) even

on saturated sands, and a reduction in erodibility (El Mountassir et al. 2021). The treatment also produced a loss in peak shear strength, with an associated reduction of dilation, an increase in critical shear stress and a reduction of the initial stiffness (Salifu and El Mountassir 2019). Each of these aspects of behaviour may have an application within geotechnical engineering, thus further research is beneficial. Salifu (2019) identified the limitation of using lignocel, as its physical form influenced the resulting stress-strain behaviour. To the best of the authors' knowledge, there are no published studies on the stress-strain behaviour of sand treated with saprotrophic filamentous fungi using powdered organic matter. Saprotrophic fungi possess the enzymes that can breakdown and utilize biopolymers (Johnson and Gehring 2007), therefore, a powdered form offers a suitable alternative to lignocellulosic fibers. The aim of this study is therefore to investigate, for the first time, the potential of harnessing synergy between saprotrophic filamentous fungi and powdered biopolymers, guar gum and xanthan gum, for soil stabilisation. Unconfined compressive strength (UCS) testing was performed to determine the stress-strain behavior of sand amended with 1% biopolymers (XG and GG) and treated with either liquid or solid form fungal inoculants. Visual assessment of mycelium growth was also performed and reported. *Pleurotus Eryngii* (black pearl king oyster mushroom), a saprotrophic, non-parasitic fungus was chosen in the present research.

## MATERIALS

**Sand:** Leighton Buzzard sand was used as the base soil. The particle size distribution of the sand was determined following BS 1377-2:1990 (1990) indicating a uniformly graded material with most particles within the range 630-860  $\mu\text{m}$ . The sand was washed on a 63 $\mu\text{m}$  sieve using tap water to remove any potential residues and the retained fraction was oven dried at 105 °C for 24hrs and left to cool to room temperature before being used.

**Fungi:** *Pleurotus Eryngii* grain spawn (sterilized and hydrated grains inoculated with a pure culture of mycelium) was procured from Merit Mushrooms UK, and stored in a fridge at <5 °C, as recommended by the manufacturer.

**Biopolymer:** Powdered guar gum and xanthan gum were purchased from Merck under the Sigma-Aldrich brand. As recommended by the manufacturer, both were stored with the lid tightly closed in a cool, dry, and well-ventilated place.

## EXPERIMENTAL WORK

A liquid fungal inoculant was envisioned as particularly suitable for large-scale field use due to its ease of application. Consequently, three fungal inoculants, one solid spawn and two liquid suspensions, were compared alongside two biopolymers, Guar Gum (GG) and Xanthan Gum (XG). Unconfined Compressive Strength (UCS) were performed to determine the mechanical behaviour of the stabilised soil samples. Simultaneously, a visual examination was performed to qualitatively assess the influence of inoculant and biopolymer type on fungal growth.

### Sample Preparation.

Initial testing of the inoculant type/inoculation method was undertaken to determine whether each would produce suitable fungal mycelium growth over a period of 14 days.

### Fungal Inoculation Methods.

Consequently, the liquid suspension inoculate was not taken forward into the next stage of testing.

### **Liquid Blend (LB).**

1g fungal spawn: 2ml water was blended in a single-serve kitchen blender for approximately 2 minutes until it formed a thin liquid consistency. The liquid was passed through a 2mm sieve to remove any solid particles. Initial testing found poor fungal growth for treatment of cylindrical sand samples when combined with GG or XG. It was observed that, the fungal growth appeared uniform but visibly weak, and it did not support soil agglomeration. Consequently, disintegration upon extrusion from the mold was observed. The poor growth may be due to the harsh blending process as physical damage to microbial cells can disrupt the integrity of the cellular membrane leading to cell death (Rangel et al. 2015). As the inoculation method produced fungal growth, albeit poor quality, liquid blend inoculate was taken forward into the next stage of testing.

### **Solid Spawn (SS).**

The spawn covered grains were broken up by hand and mixed into the sand with distilled water (1g fungal spawn : 2ml distilled water). Good growth of mycelium was found after 14 days on a cylindrical sand sample when the solid spawn was combined with 1% GG or XG. Following these results, solid spawn inoculant was taken forward into the next stage of testing.

### **Preparation of Sand-biopolymer-fungi Composite for UCS Testing.**

To produce sand-fungi-biopolymer composites suitable for UCS testing according to BS 1377-7(1990), the soil samples (Table 1) were prepared in cylindrical plastic tubes with a length to diameter ratio of 2 : 1 (dimensions: 7.8cm length and 3.6cm diameter).

**Table 1: Sample Composition.**

Component	Composition
Dry density (sand + biopolymer)	1.4g/cm <sup>3</sup>
Sand	99%
Biopolymer	1%
Water content (distilled water or liquid fungal inoculate)	10% of dry mass
Dry spawn (used in fungal inoculate if treated)	5% of dry mass

To prepare a sample, one end of the tube was covered with clingfilm (polyethylene film) and secured with an elastic band. To facilitate airflow to the sample, twenty holes were punctured into the clingfilm cover using a thin metal wire (1mm diameter). The sample composition followed Table I, and was selected based on the research and recommendations of Salifu (2019). The dry components consisted of 99% sand and 1% biopolymer. The inoculants (Solid spawn or Liquid Blend described in the above sub-section) were prepared using a ratio of 1g fungal spawn : 2ml distilled water and were applied to achieve a sample water content of 10%. The dry components were initially combined before the addition of and mixing with the fungal inoculant. The resulting mixture was lightly compacted in five layers of equal mass into the tube, achieving a dry density of 1.4g/cm<sup>3</sup>. Preliminary investigation indicated that this density was the lowest achievable within the specified tube without the mixture self-compacting to a denser state. A lower density, thus higher porosity, is desirable as it makes it easier for mycelium to penetrate through the sand structure (Harris 2003). The cylindrical samples were stored on their side in the dark at a constant temperature of 25 °C and relative humidity of 50%. A dark environment was chosen as a low light

intensity (darkness to near darkness) has been shown to facilitate superior growth of the mycelium of *Pleurotus* species (Rout et al. 2015). Moreover, light may stimulate fruiting, which is undesirable in this study. The selected temperature was recommended by Merit Mushrooms, which advises maintaining a substrate temperature within the range of 25-30 °C. Additionally, an optimization study by Salifu et al (2024) on the environmental conditions for the growth of *Pleurotus ostreatus*, a fungus of the same family and species as *Pleurotus Eryngii*, determined that a temperature of 25°C resulted in the optimal mycelium growth in terms of radius and biomass. A relative humidity of 50% was chosen to minimize any moisture loss or absorption between the sample and the surrounding air. After 14 days, cylindrical samples were vertically extruded from the plastic tube by using a wooden cylinder and the fungal growth was then visually inspected. The list of samples prepared are presented in Table 2.

**Table 2: Sample Identification.**

ID	Biopolymer	Fungal treatment	Time period (days)
S-GG-U-14	Guar gum	No-fungi	14
S-XG-U-14	Xanthan gum	No-fungi	14
S-GG-U-21	Guar gum	No-fungi	21
S-XG-U-21	Xanthan gum	No-fungi	21
S-GG-SS-14	Guar gum	Solid spawn	14
S-XG-SS-14	Xanthan gum	Solid spawn	14
S-GG-SS-21	Guar gum	Solid spawn	21
S-XG-SS-21	Xanthan gum	Solid spawn	21
S-GG-LB-14	Guar gum	Liquid blend	14
S-XG-LB-14	Xanthan gum	Liquid blend	14
S-GG-LB-21	Guar gum	Liquid blend	21
S-XG-LB-21	Xanthan gum	Liquid blend	21

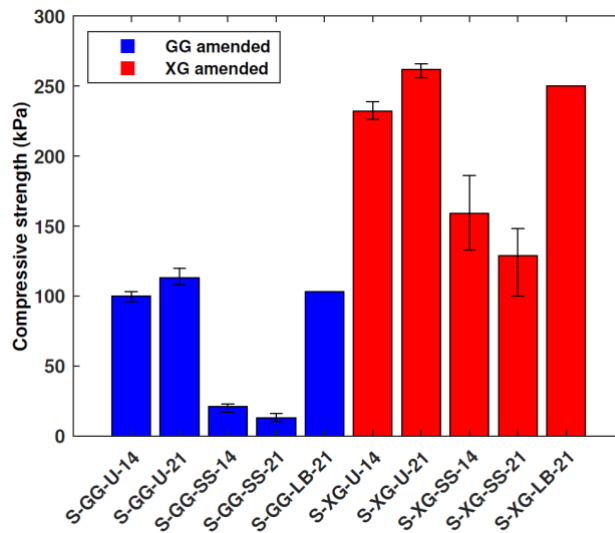
### Unconfined Compressive Testing.

Sand-biopolymer composites treated with solid spawn (SS) or liquid blend (LB) were made in triplicates following the compositions in Table 1. The samples were prepared following the method described in the previous section and were cured sealed to reduce any moisture loss. The samples were tested after curing periods of 14 and 21 days. The identification codes of each specimen composition are listed in Table 2. The 21-day growth period was selected based on recommendations from Merit Mushrooms, which advise an incubation period (the time required for the fungal mycelium to colonize and fully spread throughout the substrate) of 19-21 days. Additionally, testing the composite at 14 days provides insight into the change in geotechnical properties during fungal growth. The UCS tests followed BS 1377-7 (1990). Each sample was measured in height and diameter before being mounted and centered on the loading plate. The load piston was lowered to reach contact with the sample and the machine was zeroed. The piston compressed the sample at a rate of 1mm/min, continuously recording the force produced, and the test was stopped once the force dropped by 20% from its peak value.

## RESULTS AND DISCUSSION

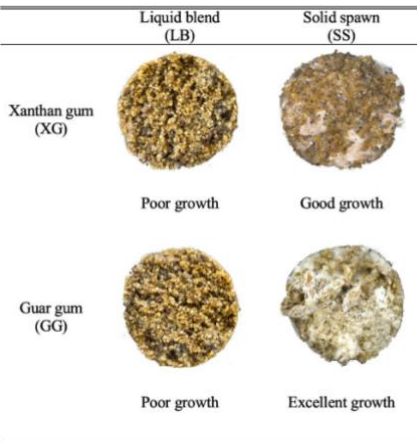
The average peak unconfined compressive strength of each sample is shown in Figure 1 and representative vertical stress/strain plots for the UCS tests are shown in Figures 3 and 4. The measurement of the unconfined compressive strength of the untreated sand was possible because

the addition of biopolymer alone provided enough cohesion for the material to stand. The biopolymer - liquid blend (LB) samples displayed less surficial growth across both 14 and 21 days. A thin growth of mycelium was found homogeneously across the samples. At 14 days, the growth was not established enough to add the necessary cohesion to extrude the sample without it breaking. At 21 days, successful extrusion only occurred for one S-GG-LB-21 and one S-XG-LB-21 sample, therefore, no averages of unconfined compressive strength could be taken. Figure 1 shows a 13% gain in strength for S-GG-U and S-XG-U samples from 14 to 21 days. This change indicates that the transition of the hydrogel into a crystalline state is occurring. The stiffness and the peak strength reached is dependent on environmental conditions, such as temperature and humidity, and the composition of the soil (Chang et al. 2015). The transformation of hydrogels to their glassy state takes 3-5 weeks and studies have reported that the majority of strengthening occurs within 7 to 28 days (Muguda et al. 2017).



**Figure 1: UCS Peak Strength.**

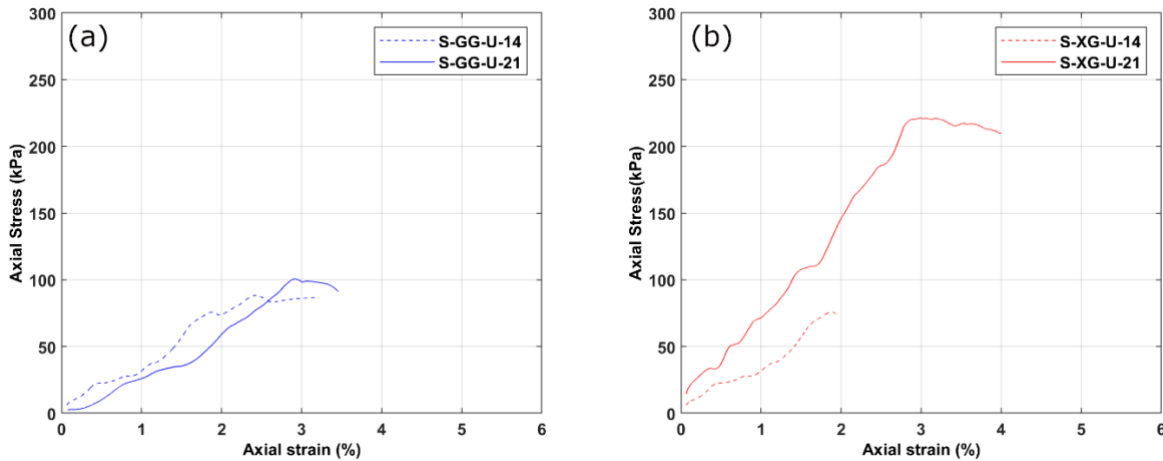
Visual observations at 14 and 21 days for solid spawn samples revealed that GG-fungi amended samples exhibited more pronounced hyphae growth than XG-fungi amended samples. Figure 2 shows the fungal growth on the outside of the samples after 21 days. S-GG-SS-21 had a higher proportion of white mycelium which is indicative of dense growth than S-XG-SS-21. Interestingly, the liquid blend samples lacked surficial growth of the mycelium, but the trend of guar gum having higher growth than xanthan gum was the same. The rate of mycelium growth is influenced by the fungus' ability to break down and utilize the biopolymer as a food source. GG is a plain polysaccharide primarily composed of mannose and galactose units linked together in a linear chain. As a simple polysaccharide, it was easier for a fungus to absorb nutrition from guar gum resulting in rapid growth of mycelium. In contrast, XG is a more complex polysaccharide with side chains and cross-links, resulting in a highly branched and intricate molecule. This branching and molecular arrangement contribute to XG high viscosity property, making it difficult for the fungi to easily nutrition. In this situation, the fungi would branch out for easily available glucose and mannose groups rather than glucuronic acid groups leading to dispersed rather than localized growth the mycelium. The unused glucuronic acid in turn would react with soil particles leading to a higher soil-to biopolymer interactions in the case of xanthan gum.



**Figure 2: Internal fungal growth.**

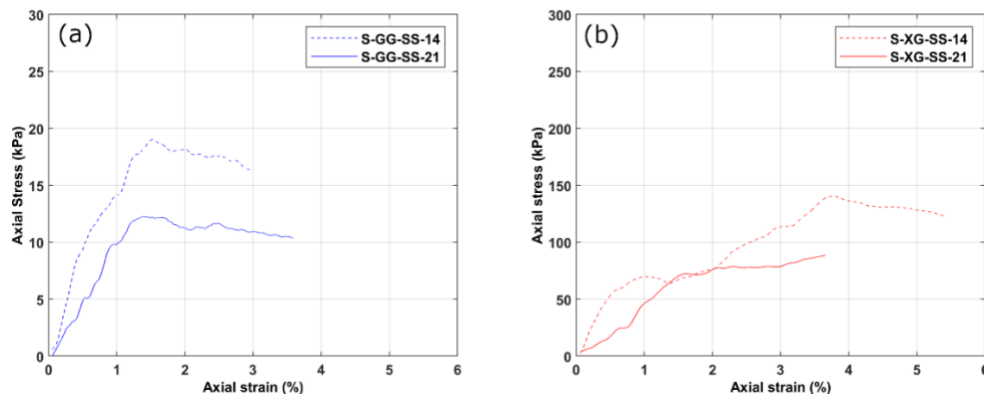
Comparisons of strength between biopolymer-fungi amended samples (SS & LB), and biopolymer amended(U) provide insights on the impact mycelium growth on the mechanical behaviour of the sample. There is substantial decrease in the strength for samples in which guar gum was introduced as a potential nutrition source. In the case of xanthan gum, though there is a significant reduction in strength, the fungi-xanthan amended samples show higher strength than all guar samples. This can be observed from the peak strengths of the achieved for all the samples. Figure 1 shows the peak strength for S-GG-U-14 was 100kPa, whereas for S-GG-SS-14, it was 21kPa (79% lower). The peak strength of S-XG-U-14 was 232kPa, while for S-XG-SS-14 it was 159kPa (31% lower). Additionally, there was a decrease in peak strength between 14 and 21 days for the biopolymer amended, solid spawn treated samples. The peak strength of S-GG-SS decreased by 8kPa (37%) and S-XG-SS decreased by 30kPa (19%). This behaviour suggests that the fungus is metabolizing both biopolymers as nutrient sources and indicates continuous fungal growth during the 14- to 21- day period. As the excess nutrition from biopolymer in the sample diminishes over time, the contribution of fungal growth to the strength becomes increasingly evident. The larger percentage differences between S-GG-U-14 and S-GG-SS-14 and between S-GG-SS-14 and S-GG-SS-21 versus its XG counterparts reinforces that GG is more easily broken down, thus a superior nutritional source for the fungi.

Figure 1 shows at 21 days, the liquid blend (LB) samples to possess strength only slightly lower than that of biopolymer amended, untreated samples (8% lower for S-GG-LB-21 and 5% lower for S-XG-LB-21). These results are to be expected for a sample exhibiting poor growth. A high biopolymer content will be present in the sample; thus, it will possess similar strength properties to its untreated counterpart. As seen in Figure 1, the range of peak strengths for repeat results of biopolymer amended, solid spawn treated samples after 14 and 21 days demonstrates a notable increase from biopolymer amended, untreated samples. This outcome was anticipated given the complex interplay of 3D fungal growth within a heterogeneous material. It is unrealistic to anticipate high quality replication during the early-stage development of fungal composite production. Furthermore, the physical characteristics of the spawn grains may have impacted the stress-strain response, emphasizing the need for future investigations to concentrate on the development of a fungal inoculant in liquid or powder form.



**Figure 3: Stress-strain behavior in UCS tests for biopolymer amended soils (a) guar gum, and (b) xanthan gum, after 14 and 21 days.**

Comparing the stress-strain graphs in Figures 3 and 4, a steep post-peak drop in stress is exhibited by just biopolymer amended samples, in contrast, solid spawn samples show a flatter drop-off, indicating a more ductile material. Biopolymer amended samples underwent semi-brittle failure, fracturing along a plane at an angle to the applied force. S-GG-SS samples failed via bulging, while S-XG-SS samples showed no visible macro surface cracks and test was stopped when strain as defined by BS 1377-2(1990) was reached. The reduction in brittle failure for biopolymer solid spawn amended samples is a promising indicator of the mycelium network aggregating and reinforcing the sand particles. The observable differences in mechanical behaviour between S-GG-SS and S-XG-SS are hypothesized from the following factors. S-GG-SS exhibits more pronounced fungal hyphae growth and a presumed lower biopolymer content. This results in a composite where applied loads break any remaining surface bonds between the GG and the sand particles, thereby transferring the load onto the mycelium network and allowing for deformation without sample crumbling. In contrast, S-XG-SS display less pronounced fungal hyphae growth and a presumed higher biopolymer content. This forms a composite where applied load is primarily transferred to the stronger direct bonds between the XG and the sand particles, leading to failure via internal micro-structural changes such as grain sliding or crushing. Liquid blend samples, with poor fungal growth, underwent the brittle failure as the biopolymer amended samples.





**Figure 4: Stress-strain behavior in UCS tests for fungi-biopolymer amended soils (a) guar gum, and (b) xanthan gum, after 14 and 21 days.**

This study underscores both the potential and limitations of using biopolymers for soil stabilization. While biopolymers alone can certainly enhance soil's mechanical properties, they are prone to strength loss when exposed to water as reported in previous studies. To address this, this study explores the synergistic use of fungi with guar and xanthan gums. Guar gum amended fungi samples, despite its rapid initial growth, eventually led to strength loss, indicating compromised long-term stability. In contrast, xanthan gum exhibited controlled mycelium growth, resulting in superior mechanical behaviour. The findings revealed that soil stabilized with just biopolymers showed higher initial strength. However, the combination of fungi and biopolymers improved cohesion and strength, benefits not observed in untreated cohesionless soil. This suggests that the fungi-biopolymer synergy not only enhances soil's mechanical properties but also provides additional advantages over untreated soils. Soils amended with fungi-biopolymer would display ductile behaviour, a characteristic not observed in soils amended solely with biopolymer. However, these fungi-biopolymer soils would have lower peak strength compared to biopolymer-amended soils. Despite this, the fungi-biopolymer synergy offers additional benefits for soil stabilization, such as inducing hydrophobicity and resistance to water ingress, making this synergistic approach a promising technique for soil stabilization requiring further studies. Nevertheless, the fungi-biopolymer approach requires more detailed investigation to optimize its efficiency for soil stabilization. Although the influence of fungal growth on the hydrophilicity of biopolymers was not investigated in this study, it is hypothesized that the durability of biopolymer-treated soil could be enhanced if mycelium growth is engineered to induce suitable levels of hydrophobicity and ductility.

## CONCLUSION

This study investigates the synergy between fungi and biopolymers in sand-fungi-biopolymer composites using unconfined compressive strength testing. Results showed that xanthan gum and guar gum enhance sand cohesion and compressive strength, with xanthan gum demonstrating superior properties. Although fungi addition reduced strength for both biopolymers, guar gum supported more pronounced mycelium growth and ductile behavior with *Pleurotus Eryngii* fungus. Further research is needed to explore the reliability of the synergy between fungi and biopolymer for soil stabilisation.

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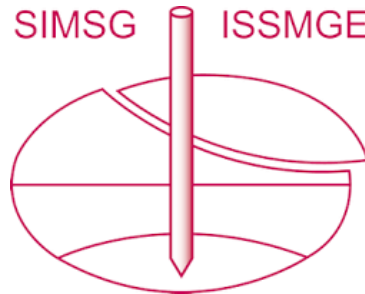
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