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Scaling up the production of myco-coagulant using solid-state fermentation for water treatment

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ABSTRACT

Aims: Providing safe drinking water is an ongoing global concern. Coagulation is an essential process in water treatment. However, most of the coagulants are chemical in nature and have negative impacts on human health and the environment. This study investigated the production of myco-coagulant in solid-state fermentation using a fungal strain.
Methodology and results: A scale-up was performed using the tray method to investigate the influence of substrate thickness (from 2-30 mm) on myco-coagulant production. The results revealed that the turbidity removal efficiency of myco-coagulant in kaolin suspension was found to be increasing with the increase in thickness of the coco peat substrate. However, the myco-coagulant extracted from the media with a thickness of 30 mm was able to remove the highest turbidity by 96%. Three different subculturing methods for mycelium inoculation were evaluated. The surface inoculation approach produced better results than other inoculation processes. The effect of initial turbidity values (50-300 NTU) on turbidity removal was studied too. The myco-coagulant was found to be the most suitable for high-turbidity water (300 NTU) with turbidity removal of 52%. Subculturing of fungus from solid-state to solid-state was also studied, which showed that the strategy was just as effective as an inoculum-based subculture.
Conclusion, significance and impact of study: Excellent bio-coagulation activity has been shown for the myco-coagulant that was isolated from the fungus strain. Subculturing using existing substrates will be more economical than subculturing using fresh inoculum. This strategy saves time, labour and cost of the coagulant production.

Keywords: Bio-coagulant, fungus, solid-state fermentation, turbidity removal

INTRODUCTION

The most common way for infections to spread is through the ingestion of contaminated or unsanitary drinking water or food. Infections cause more than 1.8 million deaths worldwide each year, with 88% of cases attributed to the intake of unsanitary water (Osiemo *et al.*, 2019). Chemicals or minerals wastes generated by various industries contain bio-hazardous and non-degradable genotoxic chemicals that contribute to water-borne infections and have ecological consequences. Unpleasant substances in effluents cause serious diseases such as reproductive impairment, cancer and neurological disorders, and help in the spread of multidrug-resistant bacteria in drinking water and surface waters (Pavankumar and Singh, 2015). Therefore, raw water must be treated before being utilized for activities and wastewater must be treated before being released to reduce pollutant levels (Kurniawan *et al.*, 2022).

Nowadays, many technologies are used to treat such noxious contaminants in wastewater. These technologies have very high efficiency in terms of treatment outcomes (Sala *et al.*, 2019). Coagulation and flocculation are part of the water and wastewater treatment units and primary treatment that can remove suspended particles (Sala *et al.*, 2019). In traditional water treatment methods, many flocculants and coagulants are used widely (Ahmed *et al.*, 2022). Two types of these materials are inorganic coagulants and synthetic organic polymers. They are all effective at removing turbidity from water (Ramavandi, 2014). Nevertheless, they are much debated owing to the usage of chemicals such as aluminium, chlorine, potassium permanganate, ferric sulphate and poly-ethylene terephthalate, which contribute to a variety of serious health problems (Ghernaout, 2018; Aghashahi *et al.*, 2020; Izabela, 2020; Li *et al.*, 2020).

The sensitivity of inorganic coagulants to water pH, as well as the risk of secondary contamination of drinking

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water with traces of toxic synthetic polymeric coagulants or residual iron and aluminium ions, are two significant challenges of water treatment processes (Bratskaya *et al.*, 2004; Ramavandi, 2014). Moreover, many studies have linked Alzheimer's disease to residual aluminium ions in treated water (Adnan *et al.*, 2017; Krupińska *et al.*, 2019). Additionally, due to the non-biodegradability of synthetic polymers, the sludge created in water treatment plants with synthetic polymers has a limited potential for recycling (Anastasakis *et al.*, 2009).

Recently, natural coagulant, which can be extracted from plant tissues, animals or microbes, has been a major focus of interest as the world moves toward green technology (Ang *et al.*, 2020). Bio-coagulants, which are mainly polysaccharides and proteins, are considered more environmentally friendly than inorganic and organic coagulants due to their biodegradability (Ramavandi, 2014). Bio-coagulants also have several advantages over chemical coagulants, including low toxicity, low residual sludge generation, and low cost (Freitas *et al.*, 2015). Bio-coagulants are therefore useful as water treatment agents and potential chemical coagulants substitutes because of their properties.

There has been much research on the use of microorganisms in wastewater treatment. However, coagulation/flocculation based on fungi is a newer technology than coagulation/flocculation based on bacteria. There is only a few research in this sector now, and they are still in their early stages. However, filamentous fungi-based coagulation/flocculation is regarded as a cost-effective and environmentally friendly technology with promising future prospects for a variety of reasons (merits of this technique) (Ahmed *et al.*, 2022). Biological flocculants have limited applicability in water treatment compared to synthetic organic flocculants since they require sophisticated extraction procedures and significant operational expenses. As a result, a bio-coagulation/bio-flocculant with low operating costs and excellent flocculation efficiency is required (Nie *et al.*, 2021).

The solid-state method has advantages over the submerged-culture method to produce fungus because it is easier to produce larger yields with high homogeneity of spores (Aidoo *et al.*, 1982). Where a solid substrate makes recovery of the product easier than in conventional liquid media (Hesseltine, 1972). Agricultural waste is a good source of carbon and nitrogen nutrients, which are good for fungal growth in solid-state fermentation (Wang *et al.*, 2019). For fungal growth, a range of substrates have been employed, ranging from rice and wheat bran to bits of wood, seeds, and roots. The choice of substrate is influenced by a number of factors, the most important of which are cost and availability (Sala *et al.*, 2019).

Coco peat substrate is a type of renewable substrate made from coconut shell processing (Lu *et al.*, 2020). The total world planted area of coconut is about 12 million ha with an annual estimated potential production of 70 billion nuts (Hoe, 2018). Coconut is Malaysia's fourth most significant industrial commodity, behind oil palm, rubber and rice. Sabah and Sarawak are the top producers of coconuts in Malaysia (Verasoundarapandian *et al.*, 2021). In Malaysia, 95% of coconut growers were smallholders with an average yield of 6,468 kg coconut/hectare in 2019. Coconut production increased from 495,531 MT to 527,729 MT from 2018 to 2019 (Nordin and Ahamad, 2021). This provides a plentiful supply of raw materials to produce coco peat as a substrate. According to the Malaysian Agricultural Research and Development Institute (MARDI), the consumption of coconut results in a significant amount of coconut residue, adding stress to Malaysia's landfill systems. A mature coconut palm produces 47-50% husks and 14-15% shell trash on average. According to Obeng *et al.* (2020), husk and shell wastes make for 62-66% of a full coconut, which might be considered a useful resource. Furthermore, coco peat prices are considered to be cheap compared to other substrates (Lu *et al.*, 2020).

Therefore, the main purpose of this study was to use the coco peat to produce a large quantity of myco-coagulants on solid-state using the tray method. The effect of substrate thickness on the coagulant production was studied. Surface inoculation, mixed inoculation, and layer inoculation were also assessed. The impact of initial turbidimetry values on turbidity removal was investigated as well. Subculturing of fungus strain from solid-state (substrate) to solid-state was also studied. SEM was used to observe the morphological structure and mycelium development of the fungus.

MATERIALS AND METHODS

Microorganisms

The fungi were obtained from the Environmental Engineering Laboratory in the Department of Chemical Engineering and Sustainability, Faculty of Engineering, International Islamic University Malaysia (IIUM), campus at Gombak, Malaysia.

Coco peat

Coco peat was purchased from a local market in Kuala Lumpur, Malaysia. It was crushed and sieved to a particle size less than 0.3 mm using a sieve shaker.

Chemical and reagents

The malt extract broth was obtained from Oxoid, Hampshire, United Kingdom. Potato Dextrose Agar was purchased from Difco™, United Kingdom. NaOH, Kaolin, D(+)-Glucose and buffer solution pH7 were acquired from R & M Chemicals, Malaysia.

Media preparation

Malt Extract Broth (MEB) and D(+)-Glucose were used in this study. To prepare 100 mL of media, 3% of Malt Extract Broth and 2.5% of glucose were mixed with

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distilled water. Then, the pH of the mixture was adjusted to 7 using NaOH prior to mixing with coco peat and autoclaving at 121 °C for 15 min.

Synthetic water preparation

In this study, kaolin was employed as a substitute for raw water in this experiment. The required amount of kaolin powder was dissolved in 10 L of distilled water to make the stock kaolin solution. With the help of a jar test apparatus, the mixture was gradually combined at 200 rpm shaking for 1 h to achieve a consistent dispersion of kaolin particles. After that, the suspension was allowed for 24 h to ensure that the kaolin was completely hydrated (Zainol *et al.*, 2021).

Fungal isolation and culture

The fungal tissues were cultured in Petri dishes with 20 mL of Potato Dextrose Agar (PDA), which was prepared according to the manufacturer's instructions. PDA was suspended in 1 L of distilled water, then heated for 1 min and thoroughly agitated to ensure that the contents dissolved completely. The mixed ingredients were autoclaved for 15 min at 121 °C before being put into sterilized Petri dishes and permitted to polymerize for at least 30 min (Galadima *et al.*, 2020). Then, the fungal strain was inoculated. Then the plates were incubated at 30 °C for 10 days to complete the mycelial growth (Figure 1). After that, the isolated fungus was purified and re-cultured in fresh media by placing a portion of mycelium in sterile Petri dishes with PDA.

Mycelial suspensions preparation

The spores were utilized to inoculate the prepared coco peat substrate. Mycelial suspensions were prepared from the culture of fungus. To do so, fungi were collected with a sterile loop by scratching the plates' media and placed inside a beaker with sterile distilled water. Beakers were shaken to separate spore aggregates. The used inoculum volume was 18%, which was subtracted from the volume of distilled water in the media.

Production of myco-coagulant

To produce the myco-coagulant, 25% of coco peat was mixed with 75% of media (pH 6). The mixture was poured into aluminium trays and covered with aluminium foil. The aluminium trays were inserted in the autoclave when the mixtures were sterilized at 121 °C for 15 min autoclave. The mixtures were poured into different sterilized plastic trays. The mixtures were poured in the trays had different depths (2-30 mm). Then, mycelial suspensions were used to inoculate the trays for further multiplication of the inoculum.

The inoculation technique was also evaluated using different methods. There were three subculturing methods investigated. Firstly, the substrate was spread in a tray (about 31 cm in length) to an average layer thickness of

Figure 1: Fungus culture on potato dextrose agar.

(about 31 cm in length) to an average layer thickness of 30 mm, then, the surface was sprayed with the inoculum. The inoculum was carefully sprayed to distribute it uniformly on the substrate surface. Secondly, the substrate and spores were thoroughly mixed before being deposited into a spreader, which was evenly distributed in sterile trays to a depth of 30 mm. Finally, an amount of substrate was spread in a tray to an average layer thickness of 10 mm, then the inoculum was sprayed on the surface, followed by two other layers of a substrate of 10 mm, and their surface too sprayed with the inoculum. All prepared trays were covered with aluminium foil and incubated at 25 ºC for 10 days.

Extraction of the myco-coagulant from the coco peat

After different days of incubation (1-10 days), 4 g of each culture was transferred into conical flasks, which were then mixed with 20 mL of buffer solution (pH 7). The flasks were agitating for 1 h at room temperature and 250 rpm. The separation of myco-coagulant from the biomass was done using the centrifuge at 9,000 rpm at 25 °C for 5 min. The supernatants were collected and used to determine their flocculating activity using the Jar apparatus method.

Evaluation of myco-coagulant using Jar test

The kaolin clay suspension method was used to determine the activity of myco-flocculation. The initial turbidity was measured using a turbidity meter. Different beakers containing 300 mL of kaolin suspension from each initial turbidimetry value were mixed with 10 mL of myco-coagulant. Then, the beakers were agitated at various mixing times and speeds, which consisted of rapid mixing (250 rpm) for 7 min and slow mixing (90 rpm) for 22 min. The suspensions were allowed to settle for 60 min after the agitation was stopped. Finally, samples were

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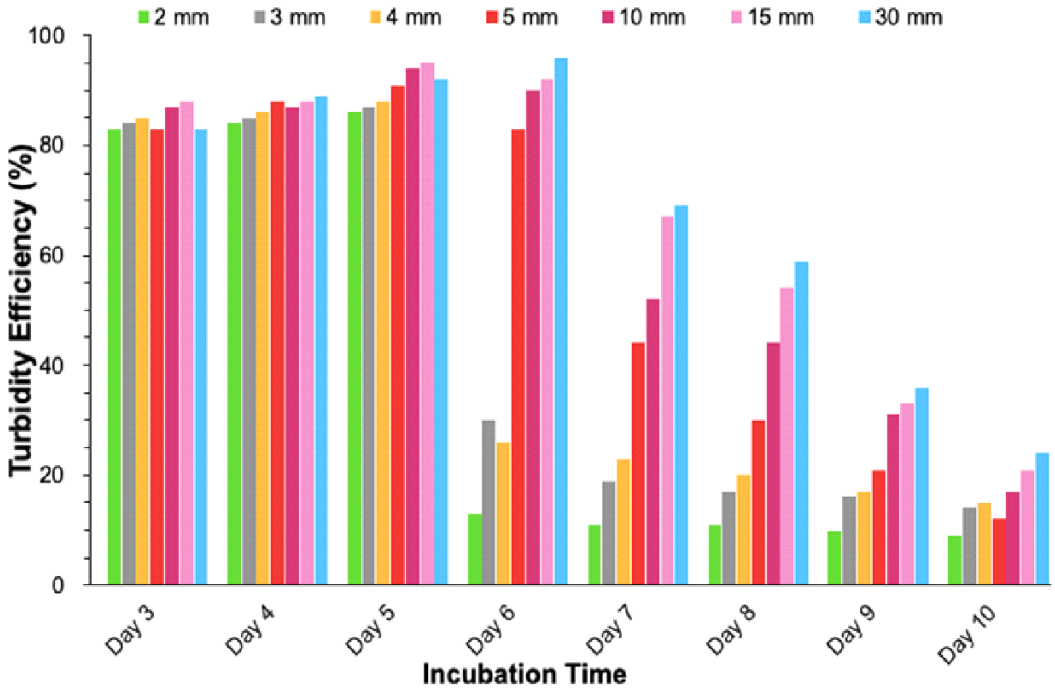

Figure 2: Removal of turbidity (%) from kaolin suspension using the myco-coagulant produced on different media depths (2-30 mm).

taken from the middle of the supernatant with a pipette to determine the final concentration. The percentage of removal was calculated to obtain the removal efficiency of turbidity, which was calculated according to the following equation:

Turbidity efficiency (%)
= [(Initial turbidity - Final turbidity)/Initial turbidity] × 100

To evaluate the effect of initial turbidimetry values on turbidity removal (%), kaolin suspensions were used with different initial turbidimetry values (50, 100, 150, 200, 250 and 300 NTU).

Sub-culture from solid-to-solid medium

In this case, the coco peat substrate that was colonized with fungus was sub-cultured from one plate to another plate. After 7 days of incubation of the fungus in the plate, around 10 g of culture was transferred into another new plate, which was prepared by mixing 25% of coco peat was mixed with 75% of Media (pH 6). The media culture depth was around 10 mm. After subculturing from solid to solid, a prepared tray was covered with aluminium foil and incubated at 25 °C for 10 days.

Scanning electron microscopy (SEM)

The morphology and the microstructure of the fungus were observed using a JEOL-IT 100 SEM instrument (JEOL, Tokyo, Japan).

RESULTS AND DISCUSSION

Assessment of turbidity removal

A produced coagulant was evaluated in terms of turbidity removal by flocculating the kaolin suspension using Jar testing, which was a primary method for assessing coagulant efficiency (Zainol *et al.*, 2021).

Effect of media depth on the production of myco-coagulant

Figure 2 illustrates the turbidity removal efficiency against the media depth (2-30 mm). Based on the results obtained in Figure 2, the turbidity removal efficiency of myco-coagulant was found to be regularly increased with the increase of the thickness of coco peat substrate within the first 5-6 days. However, the reduction of turbidity removal efficacy of myco-coagulant was shown after day 5 for all depths or day 6 for a depth of 30 mm. On day 6, with a thickness of 30 mm, myco-coagulant was able to remove turbidity by 96%.
This study had proven that the tray system was suitable to be used in SSF for myco-coagulant production using huge amounts of coco peat (substrate). The substrate thickness played one of the most important roles in achieving the highest myco-coagulant production. These results are like production by *Aspergillus niger* USM F4 via solid substrate fermentation. It found that substrate thickness played an important role in producing maximal mannanase (Rashid *et al.*, 2012).

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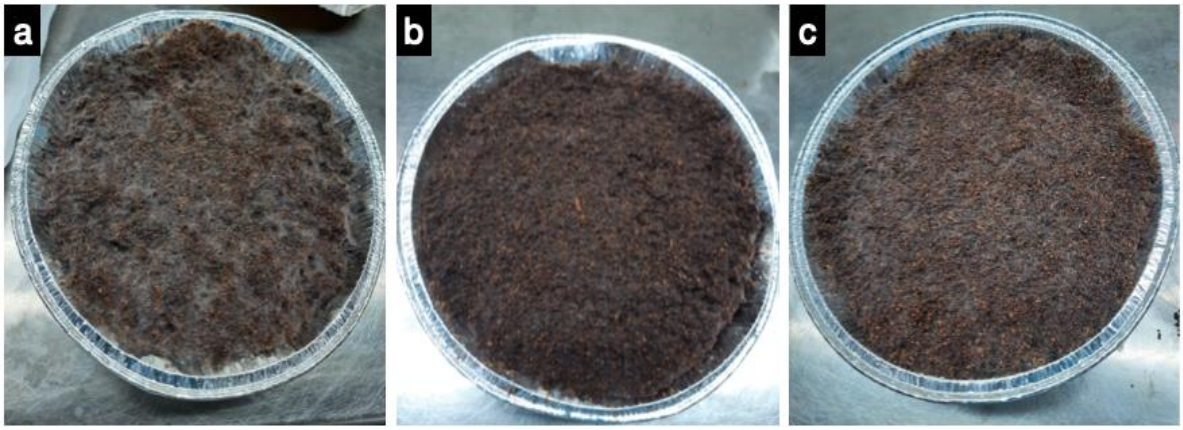

Figure 3: Fungus growth on coco peat substrate after 3 days. (a) Surface inoculation, (b) Disturbed inoculation and (c) Layer inoculation.

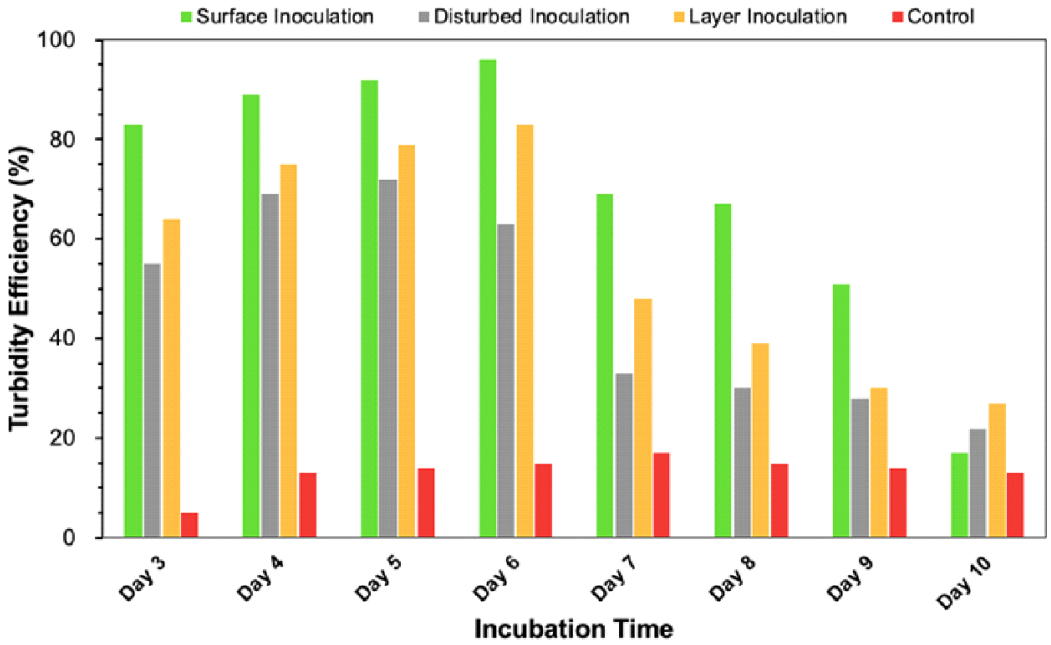

Figure 4: Removal of turbidity (%) from kaolin suspension using different subculture methods.

Effect of subculturing method on the production of myco-coagulant

Figure 3 shows that the growth of filamentous fungi occurred on the coco peat substrate surfaces. Most colonies were found in a medium that had not been disturbed with the inoculum (Figure 3a, 3c). The number of colonies on undisturbed coco peat substrate was higher than in disturbing coco peat substrate with the inoculum (Figure 3b).

The turbidity removal efficiency was plotted versus the subculturing methods in Figure 4. The results showed that myco-coagulant had a good performance in turbidity removal when coco peat substrates were not disturbed (for both surface and layer inoculations). The surface inoculation approach achieved a maximum turbidity removal of 96% on day 6. However, disturbed inoculation caused a maximum turbidity removal on day 5 with 72%, whereas layer inoculation provided a maximum turbidity removal on day 6 with 79%. After 5 days of disturbed inoculation and 6 days of undisturbed inoculation, the turbidity removal efficacy dropped to 66%, 33% and 48% for surface inoculation, disturbed inoculation and layer inoculation, respectively.

Effect of initial turbidity concentration

To assess the performance of the produced bio-flocculant, various initial turbidity concentrations were examined. The flocculation activity of the myco-coagulant

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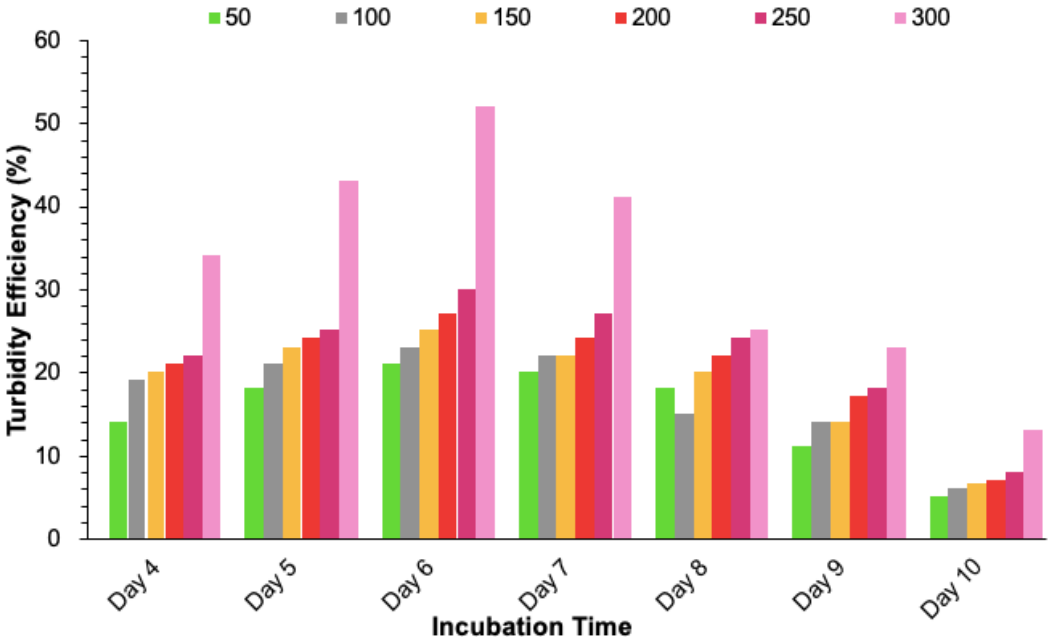

Figure 5: Removal of turbidity (%) from kaolin suspension using different initial turbidity values.

Figure 6: Subculture from solid-state to solid-state.

extracted on different days using various initial turbidity is shown in Figure 5. The bio-flocculant showed a low activity during all days on the low turbidity, whereas viewing a good performance with increasing turbidity up to 300 NTU (52%). This result suggests that the bio-flocculant produced is better suited for use in high-turbidity water than in low-turbidity water. Other researchers have reported similar findings, bio-coagulants performed better capability to remove turbidity from high turbidity compared with medium or low turbidity (Asrafuzzaman *et al.*, 2011; Ramavandi, 2014; Gaikwad and Munavalli, 2019; Kurniawan *et al.*, 2021). This is because most bio-coagulants/bio-flocculants have a mechanism for floc formation in which particle bridging becomes more efficient when more dense suspended solids are present (Kurniawan *et al.*, 2021).

Effect of subculturing from solid-state to solid-state

The subculture process was done by transferring an amount of colonized coco peat to another solid medium as shown in Figure 6. As can also be seen that the fungus has grown and colonized the whole surface of the new substrate within 3 days. Moreover, it can be observed that the colour of the solid-state that moved to a new solid-state altered after 3 days, which is due to solid media degradation during fungal growth where the natural substance acts as a nutrient supply as well as a support for fungal growth. Solid media, on the other hand, degrades during fungal development, generating changes in its physical and geometrical properties (Sala *et al.*, 2019).

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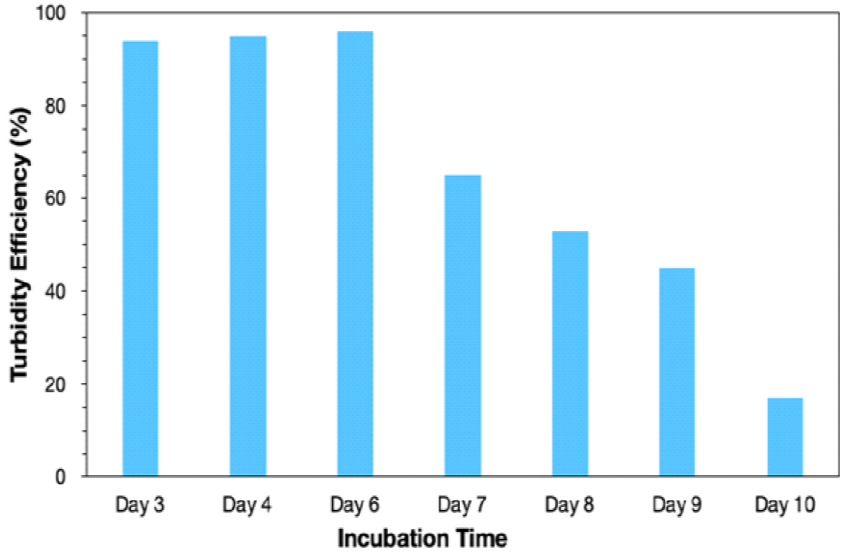

Figure 7: Removal of turbidity (%) from kaolin suspension of myco-coagulant of subculture from solid to solid.

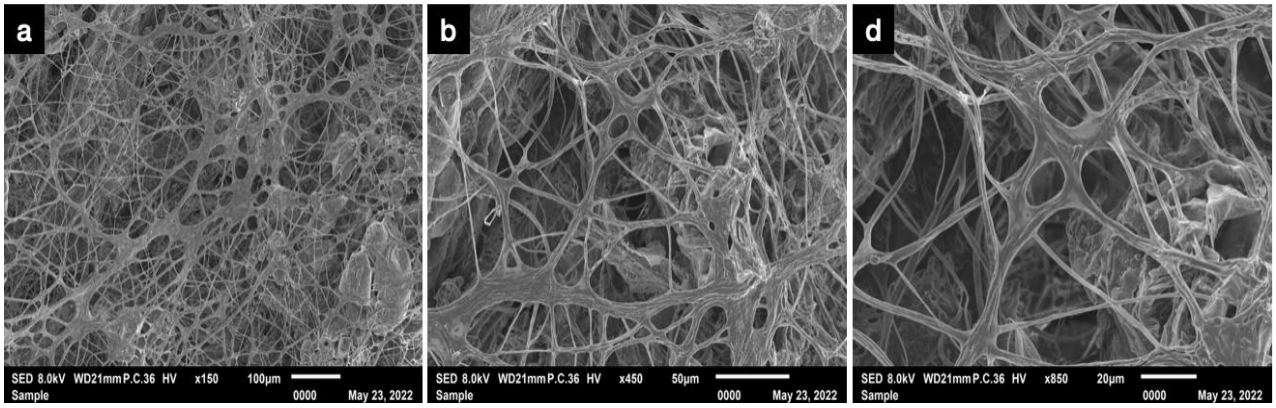

Figure 8: Scanning electron micrographs of fungus colonies on coco peat under different magnification. (a) ×150 (b) ×450 and (c) ×850 (after 10 days of incubation).

The turbidity removal efficiency of myco-coagulant extracted from solid subculturing is presented in Figure 7. The results showed that myco-coagulant had a good performance in turbidity reduction in the first 6 days. Then its performance decreased after reaching 17% on day 10. The maximum turbidity reduction of extracted myco-coagulant from solid subculturing (subculture from solid to solid) was 96%, which was like the turbidity reduction of extracted myco-coagulant from normal subculturing. These results revealed that the subculturing method was equally effective as a previous subculture that used the inoculum. In addition, this subculturing method can directly subculture fungus without mycelial suspension preparations, saving time and labor.

Scanning electron microscopy (SEM)

Scanning electron microscopy was used to analyse the morphological structure and the mycelium formation of the fungus. The micrographs of the fungus are presented in Figure 7. SEM examination displayed that the fungal mycelium was filamentous and intertwined to form a spatial network. Figure 8a shows that the coco peat substrate was fully colonized by the fungus. From Figures 8c and b, it can be seen clearly the hyphae (vegetative mycelium filaments) of the fungus, with the mean diameter of hyphae was around 0.7 µm.

CONCLUSION

The myco-coagulant isolated from the fungal strain has demonstrated excellent bio-coagulation activity and subculturing solid-state to solid-state saves time and labour, both of which are useful qualities. The novel myco-coagulant seems to be more effective for high-turbidity water. Out of three different types of inoculation process, the inoculation of the coco peat substrate using the previously grown fungus on the substrate is found to

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be more effective. Despite these results, further studies are required to improve turbidity removal using these myco-coagulants, which allows employing effectively in the coagulation and water treatment on a large scale.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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