

## ORIGINAL RESEARCH ARTICLE

# Effects of initial turbidity and myco-coagulant dose on the effectiveness of the coagulation process in water treatment

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## ABSTRACT

High turbidity is a pollutant that requires coagulants to be removed from treated water and wastewater. This study was conducted to characterize and analyze the potential of myco-coagulant-producing fungus isolated from the moist area of a kitchen. Myco-coagulant production was carried out using solid-state fermentation using coco peat as a substrate. One factor-at-a-time analysis (OFAT) was carried out to assess the capacity of the produced myco-coagulant in various initial turbidities and myco-coagulant doses. The potential of myco-coagulant was tested using turbid synthetic water with different turbidity levels (50, 100, 150, 200, 250 and 300 NTU). The results showed that turbidity removal by the myco-coagulant was influenced by the initial turbidity. The coagulant was less efficient at low turbidity levels, which was approximately 5% for 50 NTU, while the highest was 52% for 300 NTU water. Furthermore, the results demonstrated that myco-coagulant could remove the highest possible turbidities on day 6 with all initial turbidity values studied in this work. Different myco-coagulant doses ranging from 1 to 10% (v/v) were also used to determine the optimum dose for effective flocculation. The highest turbidity removal of 57% could be obtained at an optimum coagulant dose of 4% (v/v). Like any other commercial coagulant, the residual turbidity value increased at a coagulant dose higher than the optimum dose of 4% (v/v).

**Keywords:** myco-coagulant; coco peat substrate; turbidity removal; turbidity

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## 1. Introduction

Surface water resources frequently have a high level of turbidity which must be removed via flocculation or coagulation before the water is potable. Turbidity also negatively impacts consumer acceptance of water; visible cloudiness in finished water may create the perception for consumers that it is not clean or safe to drink. Turbidity is not necessarily a direct measure of microbial contamination, but microbes are often associated with particles in water<sup>[1]</sup>. Traditional water treatment methods involve the use of flocculants and coagulants<sup>[2]</sup>. These substances can be divided into synthetic organic polymers like polyacrylamide derivatives and polyethylene imine and inorganic coagulants like aluminium and ferric salts. They are effective in removing turbidity from raw water<sup>[3]</sup>. The cost of the coagulation agents plays a major role in how much it costs financially to achieve the target level of water quality<sup>[2]</sup>.

Alum coagulants are the most popular agents utilized in wastewater treatment<sup>[4]</sup> owing to characteristics that include their high effectiveness, low cost and easy availability. The use of alum salts, however, has come under scrutiny recently because of some drawbacks, including the possibility of Alzheimer's disease, the fact that they are pH-sensitive and the presence of a significant amount of residual sludge in the treated water<sup>[5,6]</sup>. As a result, several researchers are now focusing on safer coagulants that may be more efficient, safe for human health, affordable and biodegradable<sup>[6]</sup>.

During the previous few years, research has been done on different kinds of natural coagulants that come from fruit pieces like *Moringa oleifera*<sup>[7]</sup>, animals like crab, lobster<sup>[8]</sup> and shrimp shells<sup>[8,9]</sup>, bacteria like *Serratia marcescens*<sup>[10]</sup> and *Chryseobacterium daeguense*<sup>[11]</sup>, or fungi like *Lentinus squarrosulus*<sup>[12]</sup>. These substances have shown significant coagulant capacities. Where, the *Moringa oleifera* coagulant was able to achieve 87% turbidity removal from river water<sup>[13]</sup>, while the crab shell was able to remove 94.39% of turbidity from abattoir wastewater<sup>[14]</sup>. Shrimp shell was removed 83% of turbidity presented on water-based paint factory effluent (RPFE)<sup>[15]</sup>. *Serratia marcescens* bio-flocculant reached 85.45% turbidity removal from a real aquaculture effluent<sup>[16]</sup>. Bio-flocculant produced from *Lentinus squarrosulus* recorded 90% turbidity removal from kaolin suspension<sup>[12]</sup>.

Natural coagulants are considered more environmentally benign than inorganic and organic coagulants owing to their biodegradability<sup>[2]</sup>. Compared to chemical coagulants, bio-coagulants also have several benefits, such as low cost, less sludge production and low toxicity<sup>[2,17]</sup>. Due to these characteristics, bio-coagulants are advantageous as water treatment tools and prospective alternatives to chemical coagulants.

The use of microorganisms in wastewater treatment has been extensively studied. Coagulation based on fungi, however, is a more recent technological advancement than coagulation based on bacteria. A few studies have been done in this area, but they are still in their early phases<sup>[18]</sup>. However, for some reasons, filamentous fungi-based coagulation is a cost-effective and environmentally friendly technology with bright future potential<sup>[19]</sup>.

A process for bacterial or fungal culture called "solid-state fermentation (SSF)" or "bioconversion fermentation" is utilized to produce substances and products with industrial value<sup>[20]</sup>. Compared to traditional submerged fermentation (SF), SSF has several advantages, including being an easier process, consuming less energy, producing less pollution, recovering more of the product and more nearly simulating the microbes' natural habitats<sup>[21]</sup>. Microorganisms are grown in SSF using an inert or natural substrate as a solid support and a little free water or its absolute absence<sup>[21,22]</sup>. Agro-industrial residues are commonly considered the best substrates for the SSF process<sup>[23]</sup>.

One of the most popular cultivation matrices in industrial agriculture is coco peat, which is made from coconut peat. Moreover, coco peat has a significant potential for storing fertilizer and conserving water. It is regarded as the best substitute for natural peat due to its appropriate porosity and strong air permeability. It is also a type of sustainable agriculture substrate<sup>[23]</sup>.

The optimization of the coagulation/flocculation process reduces the use of chemicals and protects the environment<sup>[24]</sup>. Therefore, many research works have been done to optimize the coagulation/flocculation processes, which depend on several factors, the most relevant being initial turbidity, pH, reagents (coagulant, adjuvant) dosage and type, system hydrodynamics in coagulation and flocculation stages, temperature and alkalinity<sup>[25]</sup>.

In this study, coco peat was used to produce myco-coagulants in solid-state using the tray method from a fungal strain that was isolated from a moist kitchen area in Gombak. The effect of the operational parameters, including the myco-coagulant dose and initial water turbidity, was studied to better understand the coagulation process using the produced myco-coagulant, where the One factor-at-a-time (OFAT) analysis was performed.

Scanning electron microscopy (SEM) was also used to examine the morphological structure and mycelium development of fungi.

## **2. Materials and methods**

### **2.1. Material**

#### **2.1.1. Microorganisms**

The fungal cultures used were obtained from the environmental engineering laboratory in the Department of Chemical Engineering and Sustainability (CHES) of IIUM Gombak, Selangor, Malaysia.

#### **2.1.2. Coco peat**

The coco peat was purchased from the local market in Kuala Lumpur. Using a sieve shaker, the coco peat was crushed and sieved to a particle size of less than 0.3 mm.

#### **2.1.3. Chemical and Reagents**

Malt Extract Broth (MEB) and Potato Dextrose Agar (PDA) were acquired from Oxoid and Difco™, respectively. D(+)-Glucose, kaolin clay, buffer solution (phosphate) pH 7 and NaOH were purchased from R&M Chemical (Malaysia).

### **2.2. Methodology**

#### **2.2.1. Media preparation**

This study made use of D(+)-Glucose and MEB. 3% of MEB and 2.5% of glucose were combined with distilled water to make 100 mL of media. Then, the mixture's pH was raised to 7 using NaOH before being combined with coco peat and autoclaved for 15 min at 121 °C.

#### **2.2.2. Kaolin synthetic water preparation**

Kaolin was used in this investigation as a stand-in for water in this experiment. The stock kaolin solution was prepared by dissolving some kaolin powder in 10 L of distilled water. To produce a constant dispersion of kaolin particles, the mixture was mixed at 200 rpm for 1 hour using a jar apparatus. To ensure that the kaolin was fully hydrated, the suspension was then left to stand for 24 h<sup>[26]</sup>.

#### **2.2.3. Isolation and culture of fungus**

In Petri dishes, fungus tissues were cultivated using 20 mL of PDA, which was made in accordance with the product's instructions. PDA was suspended in 1 L of distilled water, heated for 1 min, and then vigorously stirred to ensure complete dissolution. The combined components were placed into sterilized Petri dishes and autoclaved for 15 min at 121 °C to allow the mixture to polymerize for at least 30 min<sup>[27]</sup>. After that, a fungus strain was inoculated. Later, the plates underwent a 10-day incubation period at 30 °C to finish the mycelial growth. After that, a portion of the mycelium was placed in sterile Petri dishes with PDA and the isolated fungus was purified and re-cultured in a fresh medium.

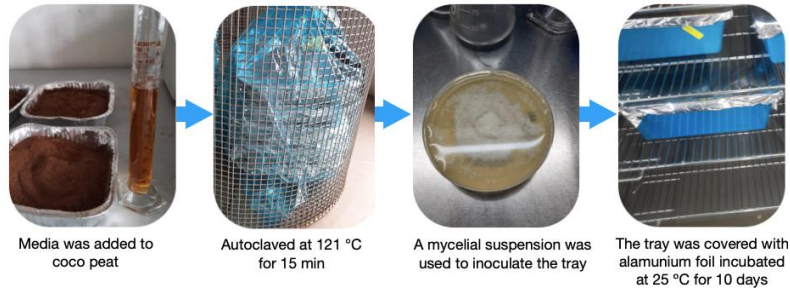
#### **2.2.4. Preparation of mycelial suspensions**

The spores were used for inoculating a prepared substrate. Mycelial suspensions were prepared from the cultures of fungus. To do this, fungi were extracted from the media of the plates using a sterile loop and put into a beaker with sterile distilled water. Spore aggregates were separated into beakers by shaking. An amount of distilled water (18%) was deducted from the total volume that was used for the media to get the inoculum volume.

#### **2.2.5. Myco-coagulant production**

To produce of the myco-coagulant, coco peat (25%) and prepared media (75%) were mixed. Then, the

mixture was poured into an aluminium tray and covered with aluminium foil. The aluminium tray was inserted in the autoclave when the mixture was sterilized at 121 °C for 15 min autoclave. The mixture was poured into a sterilized plastic tray. Then, mycelial suspension was used to inoculate the tray for further multiplication of the inoculum. A prepared tray was covered with aluminium foil and incubated at 25 °C for 10 days. The myco-coagulant production steps are presented in **Figure 1**.



**Figure 1.** Myco-coagulant production steps.

### 2.2.6. Myco-coagulant extraction

To get 10 mL of myco-coagulant extract, 4 g of culture (after ten days of incubation) were transferred into conical flasks, which were then mixed with 20 mL of buffer solution (pH 7). The flasks were stirred for 60 min at room temperature and 250 rpm. The myco-coagulant was separated from the biomass using a centrifuge running at 9000 rpm for 5 min at 25 °C. Using the Jar apparatus method, the supernatant's flocculating activity was assessed after being collected 10 mL from the supernatant.

### 2.2.7. Assessment of Turbidity Removal

**Effect of the initial turbidity concentration:** OFAT analysis was selected to assess the myco-coagulant potential. To evaluate the effect of initial turbidimetry values on the turbidity removal (%), kaolin suspensions were used with different initial turbidity values (50, 100, 150, 200, 250 and 300 NTU). A total of six different initial turbidities were prepared by diluting a kaolin stock of 700 NTU with distilled water.

The activity of myco-coagulant was assessed using kaolin clay suspension. A turbidity meter was used to measure the initial turbidity. Each 300 mL of kaolin suspension was mixed with 3% v/v of myco-coagulant in a different beaker. The beakers were then stirred for varying periods and speeds, including 7 min of rapid mixing (250 rpm) and 22 min of slow mixing (90 rpm). Then, the suspensions were allowed to settle for 60 min. All experiments were carried out at natural pH conditions without the addition of any chemicals.

To determine the final concentration, samples were obtained using a pipette from the centre of the supernatant. To determine the effectiveness of turbidity removal, the percentage of removal was determined using Equation (1).

$$Turbidity\ efficiency\ (\%) = \frac{(initial\ turbidity - final\ turbidity)}{initial\ turbidity} \times 100 \quad (1)$$

**Effect of the myco-coagulant dose:** Following the determination of the initial turbidity levels with the maximum flocculation activity, another OFAT study was conducted to measure the flocculation activity of various myco-coagulant doses. Ten different myco-coagulant dose variations (1%–10%) were evaluated. 300 mL of kaolin suspension was combined with different amounts of extracted myco-coagulant to prepare different doses (% v/v). During this step, operational settings including a 300 mL total volume in a 1000 mL beaker glass, the initial turbidity based on an earlier OFAT, rapid mixing at 250 rpm for seven minutes, slow mixing at 90 rpm for 22 min and sedimentation period of 60 min were used (**Table 1**). Using Equation (1), the flocculation activity was calculated.

**Table 1.** Operational settings.

Parameters	Values
The total volume of synthetic kaolin	300 mL
The initial turbidity	based on an earlier OFAT
Rapid mixing	250 rpm for 7 min
Slow mixing	90 rpm for 22 min
Sedimentation period	60 min

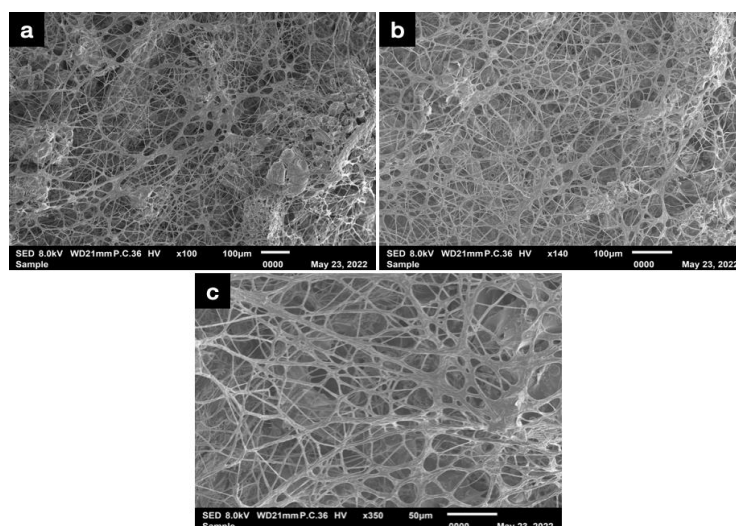
### 2.2.8. Scanning electron microscopy (SEM)

The morphology and microstructure of fungi growing on coco peat were examined using a JEOL-IT 100 SEM instrument. After incubation for 10 days, the colonies of the isolates were inspected for morphology. The sample was placed on carbon tape, transferred to a metallic stub and then left to air dry in a hood. The sample was then coated with a thin layer of gold (10-20 nm) for 50 s using a Quorum Q300TD and a 20-mA sputter current. The measurement equipment included with the JEOL IT100 SEM was used to measure cell size (InTouchScope™ version 1.060).

## 3. Results and discussion

### 3.1. Scanning electron microscopy (SEM)

The morphological structure and mycelium development of fungi were observed using scanning electron microscopy. In **Figure 2**, the fungus' micrographs are displayed. The results of the SEM analysis showed that the fungus' mycelium was filamentous and interconnected to create a spatial network. The coco peat substrate was completely colonized by the fungus, as illustrated in **Figures 2a, 2b**. The hyphae of the fungus are visible in **Figure 2c**, with a mean hyphae diameter of around 0.7  $\mu\text{m}$ . This result indicated that the coco peat was an effective substrate for fungus colonization. The main objective of the SEM imagery was to investigate the growth of fungal mycelium in the substrate, instead of finding any relation of the SEM images with turbidity removal.



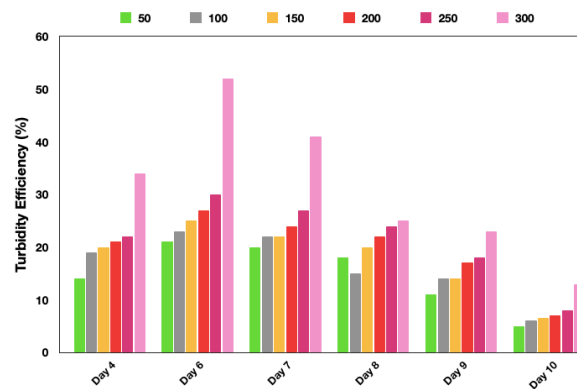
**Figure 2.** Scanning Electron Microscopy (SEM) micrographs of fungal colonies on cocopeat after 10 days of incubation (magnification of (a) 100, (b) 140, (c) 350).

### 3.2. Assessment of turbidity removal

A myco-coagulant was produced and then evaluated in terms of turbidity removal by flocculating the kaolin suspension using jar testing, which is the primary method for assessing coagulant efficiency<sup>[26]</sup>.

### 3.2.1. Effect of initial turbidity concentration

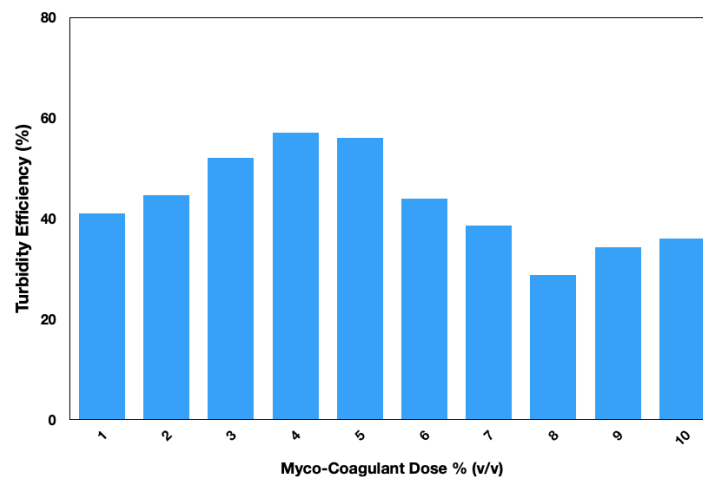
The effectiveness of the produced myco-coagulant was evaluated using a range of initial turbidity concentrations. **Figure 3** shows the flocculation activity of the myco-coagulant isolated for several days using varying initial turbidity. In general, the bioflocculant exhibited minimal activity on all days when turbidity was low, while the bioflocculant performed well when turbidity increased to 300 NTU. Furthermore, the results revealed that myco-coagulant was able to remove the highest possible turbidities on Day 6 with all differing initial turbidities. While the lowest turbidity removals were recorded on Day 10. The lowest turbidity removal was 5% which was observed at 50 NTU, while the highest turbidity removal was 52% which was obtained at 300 NTU. According to this finding, the produced bioflocculant is more suitable for usage in high-turbidity water than in low-turbidity water. Similar results have been reported by other studies, which found that biocoagulants and bioflocculants performed better in high turbidity compared to medium or low turbidity<sup>[2,10,28-31]</sup>. This is because most bio-coagulants and bio-flocculants contain a mechanism for floc formation that makes particle bridging more effective in the presence of more dense suspended particles<sup>[10]</sup>. A high level of turbidity owing to a larger particle concentration in water leads to an increase in the frequency of collisions between suspended particles and coagulants, permitting a more effective coagulation-flocculation process. According to Stoke's law, thicker and denser flocs would form with more contact and interaction between coagulating and suspending particles, increasing the rate of sedimentation throughout the treatment process<sup>[32]</sup>.



**Figure 3.** Removal of turbidity (%) from kaolin suspension using different initial turbidity values (speed mixing at 250 rpm for 7 min, slow mixing at 90 rpm for 22 min, and sedimentation period of 60 min).

### 3.2.2. Effect of myco-coagulant dose

In this study, ten myco-coagulant doses were employed to investigate its effect on turbidity removal. The results of OFAT analysis for various myco-coagulant doses are shown in **Figure 4**.



**Figure 4.** Removal of turbidity (%) from kaolin suspension using different myco-coagulant doses (speed mixing at 250 rpm for 7 min, slow mixing at 90 rpm for 22 min, and sedimentation period of 60 min).

The flocculant activity gradually increased with the increase in the myco-coagulant dose. The highest removal of turbidity of synthetic kaolin wastewater was recorded at 57% due to a dose of 4% (v/v). On the other hand, when the myco-coagulant dose was increased in the kaolin solution to 5% (v/v) and above, the turbidity removal declined. There was a significant decrease in coagulation performance from 56% to 29%, with the increase of myco-coagulant dosage from 5% to 10% (v/v), respectively. Using a higher bio-flocculant dose (more than 4%) did not increase the flocculation activity. Similar research showed that increasing the amount of flocculants did not significantly enhance the total removal and even decreased the removal of turbidity from water<sup>[2,10,28,29]</sup>. An excessive amount of flocculant will not interact with the suspended particles, leading to the formation of more suspended solids, which in turn increases turbidity<sup>[10]</sup>. The optimum doses of various types of coagulants are given in **Table 2** for comparison purposes, which also varies with the initial turbidity of the water.

**Table 2.** The optimum dose of different coagulants for various initial turbidity.

Source of Coagulant	Initial Turbidity (NTU)	The Optimum Coagulant Dose	Reference
CaCl <sub>2</sub> -PAM (calcium chloride-polyacrylamide)	353-399	2-3 mg/L	[33]
<i>Moringa oleifera</i>	50 and 150	150 mg/L	[30]
Pine cones	67 and 75	0.5 ml/L	[34]
Chitosan	200	0.5 mg/L	[35]
Graphene oxide (GO)	193.34	4.0 mg/L	[36]
<i>Lemna perpusilla</i>	300	30 mg/L	[37]
<i>Lentinus squarrosulus</i>	600	1% (v/v)	[38]
Myco-coagulant	300	4% (v/v)	This Study

## 4. Conclusion

Myco-coagulant produced by isolating white rot fungi from a wet kitchen area displayed great potential for removing turbidity from highly turbid water. The myco-coagulant performed better in high turbidity water compared with low turbidity water. Thus, the produced myco-coagulant was found to be ineffective with low turbidity water but effective with medium and high turbidity water. A myco-coagulant dose of 4% v/v was the optimum for turbidity removal. The results demonstrated that the initial turbidity had an impact on turbidity removal, while the myco-coagulant dose had no impact on the removed turbidity. SEM micrographs showed that white rot fungi effectively colonized the solid substrate. This result indicated that the coco peat was an effective substrate for fungus colonization.

## Author contributions

Conceptualization, AAM and RN; methodology, RN; formal analysis, RN, AAM and MZA; investigation, RN and AAM; resources, RN; data curation, RN and AAM; writing—original draft preparation, RN; writing—review and editing, AAM and MZA; supervision, AAM; project administration, AAM. All authors have read and agreed to the published version of the manuscript.

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## Conflict of interest

The authors declare no conflict of interest.

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