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ASSESSMENT OF POWDER MYCO-COAGULANT EXTRACTED FROM *PHANEROCHAETE CONCRESCENS* FOR WATER TREATMENT

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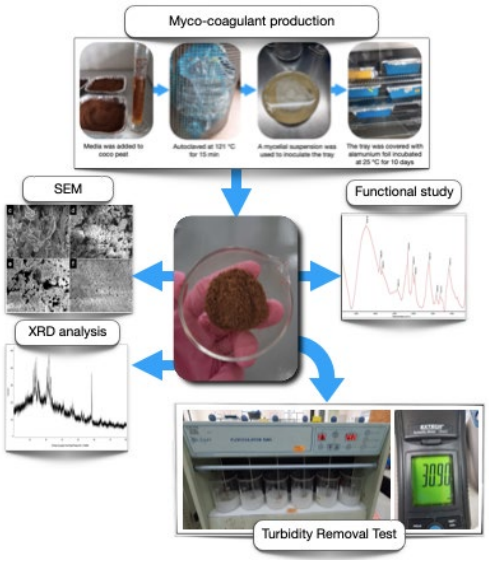
Graphical abstract



Abstract

Bio-coagulants are attracting current research interest because they are more environmentally friendly and safer than traditional chemical coagulants. However, the main challenge for bio-coagulants is the production in bulk quantities at a reasonable cost. The main purpose of this study was to investigate the ability of *Phanerochaete concrescens* to produce an effective bio-coagulant in powder form. The one-factor-at-a-time approach (OFAT) was performed to evaluate the capacity of the powdered myco-coagulant for various initial turbidities and coagulant doses. The morphological structure, functional groups and crystallinity of the bio-coagulant were evaluated using scanning electron microscope (SEM), Fourier transform infrared (FTIR) and X-ray diffractometry (XRD), respectively. The addition of 0.09-0.11 g of powder myco-coagulant led to the maximum elimination of turbidity from synthetic kaolin wastewater, which was 80 % from an initial turbidity value of 750±10 NTU. SEM revealed that the fungus that produced the coagulant has a filamentous and linked network structure. FTIR illustrated the presence of hydroxyl, carbonyl, carboxyl, methoxyl and amino groups. The XRD analysis revealed the bio-coagulant to have smaller particle sizes with wider peaks. Based on the findings, *Phanerochaete concrescens* may find use in industry as a producer of powder bio-coagulants.

Keywords: Powder Myco-Coagulant, *Phanerochaete concrescens*, Turbidity removal, Solid-State Fermentation.

1.0 INTRODUCTION

Demands for clean water are rising as the population grows. Since rivers, lakes and reservoirs still contain significant levels of contaminants such as organic compounds, heavy metals, and dangerous germs, it is not advisable to consume the water directly [1]. Human health will be negatively impacted if raw water is directly consumed by individuals. As a result, adequate treatment must be performed on both raw water and wastewater before they are released into the environment to lower their pollutant contents [2].

Various conventional and advanced methods have been used to clean water and wastewater. Coagulation is a crucial physicochemical process that has been widely used as a pre-or post-process step in wastewater treatment. The major purpose of coagulation is typically to reduce turbidity by removing colloidal elements like microorganisms from water and wastewater [3]. Chemical coagulants employed in a variety of procedures for the purification of drinking water might pose serious environmental and health problems [4,5]. Consequently, bio-coagulants have lately drawn interest on a global level [6].
Bio-coagulants may be extracted from plant tissues, animals, or microbes [6]. They are mainly polysaccharides and proteins [7].

Bio-coagulants provide a variety of advantages over chemical coagulants, including reduced sludge generation and low toxicity [8]. The use of microorganisms in wastewater treatment has been extensively studied. Coagulation/ flocculation based on fungus, however, is a more recent technological advancement than coagulation/flocculation based on bacteria. Only a few studies are currently being conducted in this field, and they are only beginning. However, for several reasons, coagulation/flocculation using filamentous fungus is viewed as a cost-effective and eco-friendly benign method with excellent prospects (merits of this technique). Even though, commercial production of myco-coagulant is not reported yet, let alone the information about the production of coagulant using *Phanerochaete concrescens* [9].

Therefore, this study aimed to extract bio-coagulant from *Phanerochaete concrescens* and convert it to powder form. The coagulation activity of myco-coagulant powder was also assessed using a one-factor-at-a-time approach (OFAT), where various myco-coagulant powder doses (0.01-0.14 g) and initial turbidities (150±10 - 750±10 NTU) were studied. SEM was used to observe the morphological structure of the fungus, myco-coagulant, flocculated and un-flocculated kaolin clay. FT-IR and XRD were also used to evaluate the functional groups and crystallinity of the bio-coagulant, respectively.

2.0 METHODOLOGY

2.1 Microorganism and Chemicals

Phanerochaete concrescens was obtained from the Chemical Engineering & Sustainability Department, International Islamic University Malaysia (IIUM). Malt Extract Broth (MEB) was obtained from Oxoid. Potato Dextrose Agar (PDA) was purchased from Difco™. Sodium hydroxide (NaOH), Kaolin, D(+)-Glucose, and Buffer solution of pH7 were acquired from R&M Chemicals.

2.2 Cocopeat

Cocopeat was used in solid-state fermentation as a source of carbon for fungal growth. Coco peat was collected from IIUM, Kuala Lumpur. It was crushed and filtered to a particle size less the 0.3 mm using a sieve shaker [9].

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

2.4 Fungal Isolation and Culture

The fungi were isolated from a moist area in the kitchen. 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 minute and thoroughly agitated to ensure that the contents dissolved completely. The mixed ingredients were autoclaved for 15 minutes at 121°C before being put into sterilized Petri dishes and permitted to polymerize for at least 30 minutes [10]. Then, the fungal strain was inoculated. After that, the plates were incubated at 30 °C for 10 days to complete the mycelial growth. Next, the isolated fungus was purified and re-cultured in new media by placing a piece of mycelium in sterile PDA Petri plates.

2.5 Mycelial Suspensions Preparation

The spores were utilized to inoculate a prepared substrate. Mycelial suspensions were prepared from the cultures of fungus. To achieve this, fungi were collected from the media plates using a sterile loop and put in a beaker with sterile distilled water. Beakers were shaken to separate the spore. The used inoculum volume was 18 %, which was subtracted from the volume of distilled water of the media [9].

2.6 Production of Myco-Coagulant

For the production of the myco-coagulant, 25 % of coco peat was mixed with 75 % of media (pH 7). The mixture was poured into an aluminium tray and covered with aluminium foil. The aluminium tray was inserted in the autoclave when the mixtures were sterilized at 121 °C for 15 mins autoclave. Then, the substrate was spread in a sterilized tray to an average layer thickness of 30 mm. After that, the surface was sprayed with the inoculum. The inoculum was carefully sprayed to distribute it uniformly on the substrate surface. A prepared tray was covered with aluminium foil and incubated at 30 °C for 10 days [9].

2.7 Extraction and Purification of Myco-Coagulant

After 7 days of cultivation, the fermented medium of solid-state fermentation was mixed with a buffered solution pH 7.0. The mixture was agitated in a rotary shaker for 1 hour at 250 rpm at 30 °C. Then, it was filtered using muslin and Whatman paper. The separation of myco-coagulant was performed by centrifuging culture suspension (buffer solution + mycelium) at 9000 rpm at 27 °C for 10 min and the supernatant was collected. Then, the supernatant was mixed with four volumes of cold ethanol (95%) at a ratio of 1:4 (Figure 1a).

To allow for sedimentation, the mixture was left undisturbed for 48 hours. The supernatant and the myco-coagulant were separated by centrifuging it at 9000 rpm for 30 minutes. The residue re-dissolved in distilled water at a ratio of 1:4 (v/v). The process was twice sequentially performed, and the myco-coagulant that had been purified was lyophilized and freeze-dried (Figure 1b). The lyophilized fraction was crashed and sieved (Figure 1c) for use in further studies [11]. Various amounts of dry powdered myco-coagulant were mixed in distilled water to produce coagulant doses ranging from 300 to 367 mg/L.

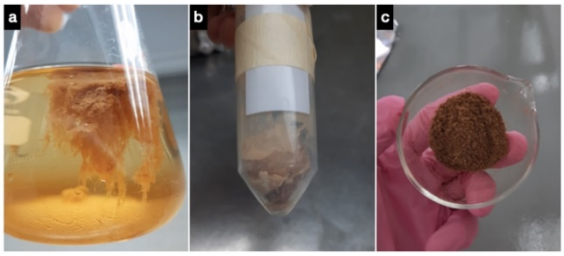


Figure 1 (a) The extract of fungal cultivate mixed with cold ethanol, (b) dried myco-coagulant, and (c) crashed and sieved dried myco-coagulant.

2.8 Evaluation of Produced Bio-Coagulant Using Jar Test

2.8.1 Effect of the Initial Turbidity Concentration

Kaolin suspensions were utilized with various initial turbidity values to assess the impact of initial turbidimetry values on the turbidity removal (150, 250, 350, 450, 550, 650 and 750 NTU). By adding distilled water to kaolin stock, seven distinct initial turbidities were prepared. The activity of bio-coagulant was assessed using the kaolin clay suspension method. The turbidimeter was used to measure the initial turbidity. Each initial turbidimetry value of 300 mL of kaolin suspension was mixed with 10 mL of bio-coagulant in a different beaker. The beakers were then stirred for varying periods and speeds, including 7 minutes of speed mixing (250 rpm) and 22 minutes of slow mixing (90 rpm). Then, the suspensions were allowed to settle for 60 minutes [9]. All experiments were carried out at neutral pH conditions without the addition of any chemicals. To determine the final concentration, samples were obtained using a pipette from the top of the supernatant. The removal effectiveness was determined using Equation (1).

Turbidity removal efficiency (%) = (initial turbidity – final turbidity) x 100 / initial turbidity (1)

2.8.2 Effect of the Bio-Coagulant Dose

A second investigation was carried out to test the flocculation activity of various bio-coagulant doses. Fourteen different bio-coagulant dose variations (0.09 - 0.11 g) were evaluated. Operational conditions employed during this stage were 300 mL in 500 mL beaker glasses, the initial turbidity based on an earlier investigation, fast mixing and slow mixing at 250 rpm for 7 mins and 90 rpm for 22 mins, respectively, and a settling time of 60 mins.

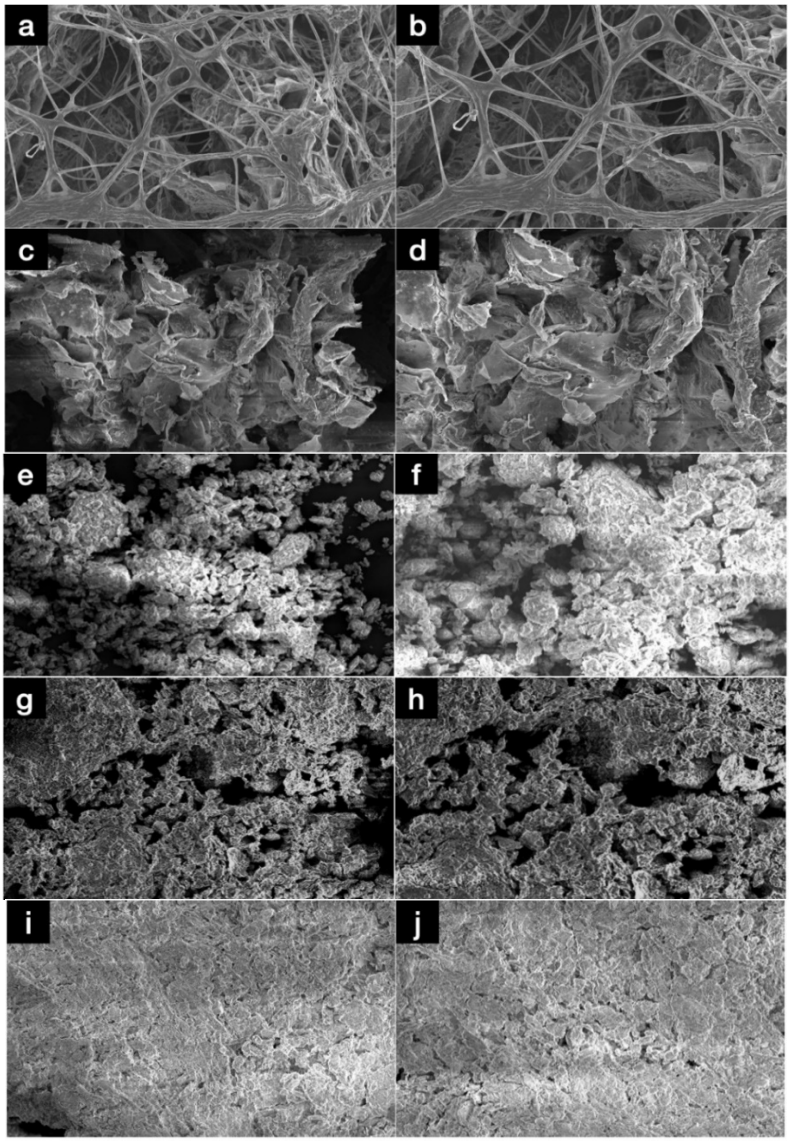


Figure 2 Scanning Electron Micrographs of Fungi on coco-peat (a) 500X and (b) 750X; Dried Myco-Coagulant (c) 500X, (d) 750X; Raw Kaolin (e) 500X, (f) 750X;, Kaolin after the flocculation process (g) 500X, (h) 750X; and Myco-Coagulant–Kaolin after the flocculation process (i) 500X, (j) 750X.

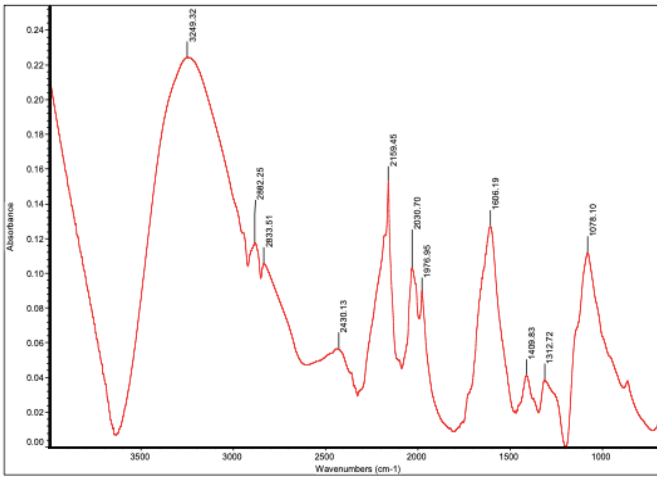


Figure 3 FT-IR spectrum of purified myco-coagulant.

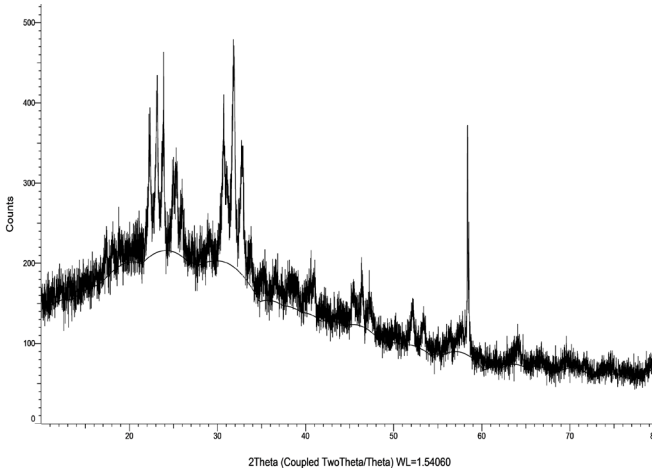


Figure 4 X-ray diffraction (XRD) patterns dry purified myco-coagulant.

2.9 Characterization of Myco-Coagulant

2.9.1 Scanning Electron Microscopy (SEM)

A fungus was put on carbon tape and coated in a gold-coating chamber, using Quorum Q300TD. Scanning electron microscopic (SEM) micrographs of a gold-coated fungus were acquired using JEOL-IT100 SEM instrument (JEOL, Japan). The micrographs of purified myco-coagulant flocculated and un-flocculated Kaolin clay samples were obtained using similar techniques.

2.9.2 Fourier Transform Infrared (FT-IR)

Fourier transform infrared (FT-IR, Thermo Scientific Nicolet™ model iS50 FTIR Spectrometer) was utilized to analyze the surface chemistry of purified myco-coagulant over wave range 400 to 4000 cm^{-1}.

2.9.3 XRD analysis

X-ray Diffraction (XRD) analysis was performed utilizing Bruker D2 Phaser (Bruker AXS, Karlsruhe, Germany) diffractometer equipped with a copper anode (Cu Kα = 1.54184 Å). A throughout a scanning interval (2θ) between 0 and 80 degrees at a scan speed of 0.25 s/step.

3.0 RESULTS AND DISCUSSION

3.1 Characterization Of Fungus

3.1.1 Scanning Electron Microscopy (SEM)

The morphological structure of fungus grown on cocopeat, powder myco-coagulant, raw kaolin clay particles, and kaolin clay suspension flocculated with/without myco-coagulant was examined using scanning electron microscopy and Figure 2 displays the findings.

Figure 2 (a, b) shows fungus grown on the coco peat substrate. According to SEM examination, fungal mycelium was filamentous and linked to create a spatial network. The hyphae of the fungus (vegetative mycelium threads) seem to have an average diameter of around 0.7 µm.

About 1.25 g of purified myco-coagulant was recovered from 1 L of myco-coagulant supernatant. According to Figure 1, the purified myco-coagulant after freeze-frying was brownish (b). The powder myco-coagulant has an irregular structure of a compact nature, as can be observed in Figure 2. c, d. The myco-coagulant's effectiveness may be attributed to its structural makeup.

The kaolin clay particles seemed small and scattered before the flocculation procedure, and no floc particles were seen (Figure 2. e, f). Small floc particles were shaped during the flocculation process in the absence of myco-coagulant (Figure 2. g, h). However, the addition of powder myco-coagulant significantly enhanced the size of the floc particles (Figure 2. i, j).

The adherence to the kaolin clay particle was accomplished by the functional moieties in the chain of the purified myco-coagulant. Due to the interaction between the myco-coagulant and kaolin clay particle, flocs were formed. These flocs eventually gathered to form bigger flocs, which gravitationally precipitated out of the suspension (Figure 2.f). This finding illustrated how crucial bridging is to the flocculation process [12]. These findings support earlier research for the pure bio-coagulants. These results are consistent with past studies on pure bio-coagulants [11–13].

3.1.2 Surface Chemistry (FTIR spectroscopy)

The purified myco-coagulant FTIR spectrum, shown in the Figure 3, exhibited numerous peaks. The broad band at 3249.32 cm^{-1} was ascribed to the existence of hydrogen-bonded (O–H) groups, which confirmed the presence of polysaccharide moiety [14], the proteins, fatty acids, carbohydrates, lignin [14] pectin and cellulose [16]. The peaks around 2882.25 cm^{-1} and 2833.51 cm^{-1} are assigned to the symmetric and asymmetric stretching of group C-H-CH_2 present in fatty acids [15]. A weak C–H stretching band was found at 2430.13 cm^{-1} [17].

The signal at 1606.19 cm^{-1} denoted the presence of a strong carbonyl group [14], indicating characteristic vibrations of the C=O stretching in the –CONH– group in proteins and amino-sugars [16], which can be attributed to stretching and bending

vibration mode in the amide I and amide II of the fungal pellet wall structure. Furthermore, the glycosaminoglycans and proteins found in the fungal cell wall, including N-acetylglucosamine and galactosamine, may have been the source of N-H groups [18]. The stretching vibration at 1078.10 cm^{-1} was indicative of the presence of pyranoside form [14]. The presence of β-glycosidic linkages between the sugar monomers is indicated by a small absorption band at 854.81 cm^{-1} [17].

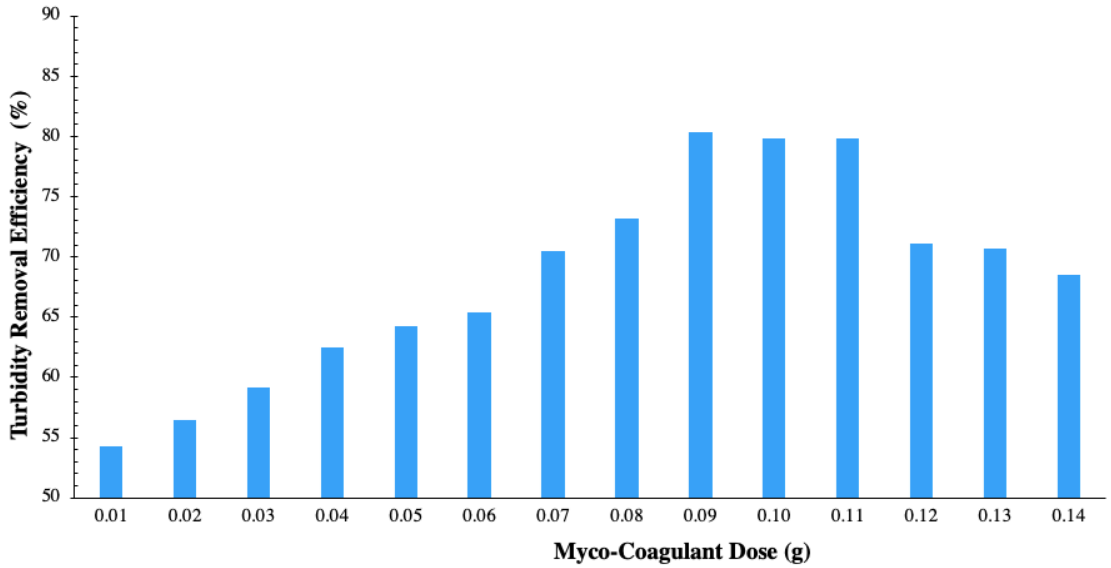


Figure 5 Removal of turbidity (%) from kaolin suspension using different myco-coagulant doses (g).

According to previously published research, the primary functional groups that contribute to the coagulation-flocculation process are the hydroxyl, carbonyl, carboxyl, methoxyl, and amino groups. The electrostatic attraction may be strengthened to offer a stronger bridging mechanism when carboxyl and hydroxyl groups are present during wastewater treatment, which ultimately improves the effectiveness of the water treatment process [12].

3.1.3 XRD Analysis

Figure 4 shows the X-ray pattern myco-coagulant representation at an angle (2θ), with deep peaks seen between 10 and 80 degrees.

The myco-coagulant has prominent peaks that are quite intense, which could indicate the presence of impurities. The particle size effect is typically responsible for the widening of peaks in a solid XRD pattern. Smaller particle sizes are indicated by wider peaks; hence the produced myco-coagulant contains small particles [19].

3.2 FLOCCULATION TEST OF POWDER MYCO-COAGULANT

3.2.1 Effect of Myco-Coagulant Dose

Fourteen different doses of myco-coagulant were used in the current investigation to examine its effect on turbidity removal. The results are shown in Figure 5.

As can be observed, the powder myco-coagulant activity increased as the myco-coagulant dosage did as well. The addition of 0.09 to 0.11 g of powder myco-coagulant led to the maximum elimination of turbidity from synthetic kaolin wastewater, which was equivalent to 300 to 367 mg/L of raw powdered coagulant dose. On the other hand, turbidity removal decreased as the myco-coagulant dose was raised in the treatment of kaolin solution to 0.1 g and above. With the increase in myco-coagulant dose from 0.01 to 0.09 g, there was a considerable increase in the performance of coagulation, from 54 to 80 %. Based on the zeta potential of the kaolin water and solution mixed with the myco-coagulant the main mechanism of turbidity removal is charge neutralisation followed by particle bridging which helped settle the kaolin particles.

Using larger amounts of powder myco-coagulant (more than 0.11) has little or no impact on flocculation activity. Similar studies revealed that adding more flocculants had no effect on the overall reduction of turbidity from water, and even had the opposite effect [7, 20–22]. A coagulant used in excess will not help flocculate the suspended particles due to the reversal of the particle charges, which would raise turbidity [22].

3.2.2 Effect of Initial Turbidity

Various initial turbidity concentrations were used to assess the performance of the powder myco-coagulant. Figure 6 illustrates the flocculation activity of the powder myco-coagulant on turbidity removal.

Overall, it can be shown that the powder myco-coagulant operated effectively when the turbidity reached 750 NTU, but it showed performance when the turbidity was low. The outcomes also showed that powder myco-coagulant could eliminate the maximum turbidity from the highest initial turbidity. The maximum turbidity removal of 79.6% was achieved for 750±10 NTU water with a residual turbidity of 153 NTU; while the lowest turbidity removal was seen for 150±10 NTU were, where it was 32.7% with a residual turbidity of 101 NTU.

These findings suggest that high turbidity water is a better application for the powder myco-coagulant than low turbidity water. Other studies that indicated that bio-coagulants and bio-flocculants functioned better in high turbidity than in medium or

low turbidity have also reported similar findings [7,15,20–23]. This is due to the mechanism for floc formation seen in the majority of bio-coagulants and bio-flocculants, which increases the effectiveness of particle bridging in the presence of denser suspended particles [22].

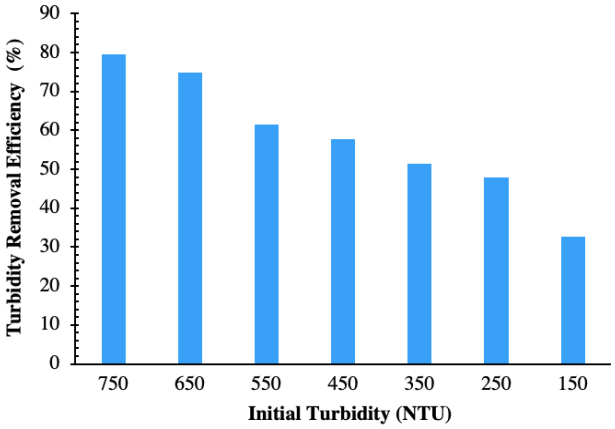


Figure 6 Removal of turbidity (%) from kaolin suspension for various initial turbidity values.

Detailed characteristics of the treated water were not determined in this study. There are plans to characterise the treated water to know the residual components of the coagulant, which requires various chemical analyses. However, the physical properties of the settled sludge are shown in Figure 2 of this paper.

4.0 CONCLUSIONS

Powder myco-coagulant isolated from *Phanerochaete concrescens* showed promising potential in reducing turbidity from highly turbid water. The performance of powder myco-coagulant was better in high turbidity water. As a result, it was revealed that powder myco-coagulant was efficient in medium and high turbidity water but less effective in low turbidity water. The turbidity removal was influenced by both the initial turbidity and powder myco-coagulant doses influenced turbidity removal. The results showed that *Phanerochaete concrescens* can be used as a source of a bio-coagulant that can be used as powder. However, compared to the liquid myco-coagulant (the supernatant) the powdered form of the coagulant exhibited 10 to 15% less efficiency, indicating that some of the coagulation ability is lost due to the drying process of the myco-coagulant.

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Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper

References

[1] Kurniawan, S. B., Imron, M. F., Chik, C. E. N. C. E., Owodunni, A. A., Ahmad, A., Alnawajha, M. M., Rahim, N. F. M., Said, N. S. M., Abdullah, S. R. S., Kasan, N. A., Ismail, S., Othman, A. R., & Hasan, H. A. 2022. What compound inside biocoagulants/bioflocculants is contributing the most to the coagulation and flocculation processes? *Science of The Total Environment* 806. DOI : https://doi.org/10.1016/j.scitotenv.2021.150902.

[2] Sharma, S., & Bhattacharya, A. 2017. Drinking water contamination and treatment techniques. *Applied Water Science.* 7 1043–67. https://doi.org/10.1007/s13201-016-0455-7.

[3] Adnan, O., Abidin, Z. Z., Idris, A., Kamarudin, S., & Al-Qubaisi, M. S. 2017. A novel biocoagulant agent from mushroom chitosan as water and wastewater therapy. *Environmental Science and Pollution Research.* 24 20104–12. DOI : https://doi.org/10.1007/s11356-017-9560-x.

[4] Nedjai, R., Al-Mamun, A., & Alam, M. Z. 2024. Effects of initial turbidity and myco-coagulant dose on the effectiveness of the coagulation process in water treatment. *Applied Chemical Engineering*, *7*(2): 1546-1546. DOI: https://doi.org/10.59429/ace.v7i2.1546

[5] Ahmad, N. S. B., Mamun, A. A., & Nedjai, R. 2023. Assessment of expired coagulant for water treatment. In *AIP Conference Proceedings*. 2713(1). AIP Publishing. DOI : https://doi.org/10.1063/5.0129719.

[6] Ang, T-H., Kiatkittipong, K., Kiatkittipong, W., Chua, S-C., Lim, J. W., Show, P-L., Bashir, M. J. K., & Ho, Y-C. 2020. Insight on Extraction and Characterisation of Biopolymers as the Green Coagulants for Microalgae Harvesting *Water*. 12(5): 1–31.

[7] Ramavandi, B. 2014. Treatment of water turbidity and bacteria by using a coagulant extracted from Plantago ovata *Water Resources and Industry* 6: 36–50. DOI: https://doi.org/10.1016/j.wri.2014.07.001.

[8] Freitas, T. K. F. S., Oliveira, V. M., de Souza, M. T. F., Geraldino, H. C. L., Almeida, V. C., Fávaro, S. L., & Garcia, J. C. 2015. Optimization of coagulation-flocculation process for treatment of industrial textile wastewater using okra (A. esculentus) mucilage as natural coagulant *Industrial Crops and Products*. 76: 538–44. DOI: https://doi.org/10.1016/j.indcrop.2015.06.027.

[9] Al Mamun, A., Nedjai, R., and Alam M. Z., 2023. Scaling up the production of myco-coagulant using solid-state fermentation for water treatment. *Malaysian Journal of Microbiology*, 19(6): 777-785. DOI: http://dx.doi.org/10.21161/mjm.230025

[10] Galadima, A. I., Salleh, M. M., Hussin, H., Mohd Safri, N., Mohd Noor, R., Chong, C. S., Yahya, A., Mohamad, S. E., Abd-Aziz, S., Yusof, N. N. M., Abu Naser, M., & Al-Junid, A. F. M. 2020. One-Step Conversion of Lemongrass Leaves Hydrolysate to Biovanillin by Phanerochaete chrysosporium ATCC 24725 in Batch Culture *Waste and Biomass Valorization.* 11: 4067–80. DOI : https://doi.org/10.1007/s12649-019-00730-w.

[11] Nwodo, U. U., Green, E., Mabinya, L. V., Okaiyeto, K., Rumbold K., Obi, L. C., & Okoh, A. I. 2014. Bioflocculant production by a consortium of Streptomyces and Cellulomonas species and media optimization via surface response model *Colloids Surfaces B Biointerfaces.* 116. 257–64. DOI: https://doi.org/10.1016/j.colsurfb.2014.01.008.

[12] Okaiyeto, K., Nwodo, U. U., Mabinya, L. V., Okoli, A. S., & Okoh, A. I. 2015. Characterization of a bioflocculant (MBF-UFH) produced by bacillus sp. AEMREG7 *International Journal of Molecular Sciences.* 16: 12986–3003. https://doi.org/10.3390/ijms160612986.

[13] Mabrouk, M. E. 2014. Production of bioflocculant by the marine actinomycete Nocardiopsis aegyptia sp *Life Science Journal* 11: 27–35

[14] Arunkumar, P., Sadish Kumar, V., Saran, S., Bindun, H., & Devipriya, S.

P., 2019. Isolation of active coagulant protein from the seeds of Strychnos potatorum–a potential water treatment agent *Environmental Technology*. 40(12): 1624–1632. DOI: https://doi.org/10.1080/09593330.2018.1427798.

[15] Baptista, A. T. A., Silva, M. O., Gomes, R. G., Bergamasco, R., Vieira, M. F., & Vieira, A. M. S. 2017. Protein fractionation of seeds of Moringa oleifera lam and its application in superficial water treatment *Separation and Purification Technology*. 180: 114–24. DOI : https://doi.org/10.1016/j.seppur.2017.02.040.

[16] Zainol, N. A., Goh, H. T., & Syed, Zainal, S. F. F. 2021. Effectiveness of Mushroom (Pleurotus Pulmonarius) Waste as Natural Coagulant for Kaolin Synthetic Water via Coagulation-Flocculation Process *IOP Conference Series: Earth and Environmental Science*. 920. DOI: https://doi.org/10.1088/1755-1315/920/1/012020.

[17] Pathak, M., Sarma, H. K., Bhattacharyya, K. G., Subudhi, S., Bisht, V., Lal, B., & Devi, A. 2017. Characterization of a novel polymeric bioflocculant produced from bacterial utilization of n-hexadecane and its application in removal of heavy metals *Frontiers in microbiology* 8: 1–15. DOI : https://doi.org/10.3389/fmicb.2017.00170.

[18] Nie, Y., Wang, Z., Zhang, R., Ma, J., Zhang, H., Li, S., & Li, J. 2021 Aspergillus oryzae, a novel eco-friendly fungal bioflocculant for turbid drinking water treatment *Separation and Purification Technology*. 279: 119669. DOI: https://doi.org/10.1016/j.seppur.2021.119669.

[19] Tsilo, P. H., Basson, A. K., Ntombela, Z. G., Maliehe, T. S., & Pullabhotla, R. V. S. R. 2021. Isolation and Optimization of Culture Conditions of a Bioflocculant-Producing Fungi from Kombucha Tea SCOBY *Microbiology Research*. 12: 950–66. DOI : https://doi.org/10.3390/microbiolres12040070.

[20] Gaikwad, V. T. & Munavalli, G. R. 2019. Turbidity removal by conventional and ballasted coagulation with natural coagulants *Applied Water Science* 9: 1–9. DOI: https://doi.org/10.1007/s13201-019-1009-6.

[21] Asrafuzzaman, M., Fakhruddin, A. N. M., & Hossain, M. A. 2011. Reduction of Turbidity of Water Using Locally Available Natural Coagulants *ISRN Microbiol.* 2011: 1–6. DOI : https://doi.org/10.5402/2011/632189.

[22] Kurniawan, S. B., Abdullah, S. R. S., Othman, A. R., Purwanti, I. F., Imron, M. F., Ismail, N. I., Ahmad, A., & Hasan, H. A. 2021. Isolation and characterisation of bioflocculant-producing bacteria from aquaculture effluent and its performance in treating high turbid water *Journal of Water Process Engineering*. 42: 102194. DOI: https://doi.org/10.1016/j.jwpe.2021.102194.

[23] Nkurunziza, T., Nduwayezu, J. B., Banadda, E. N., & Nhapi, I. 2009. The effect of turbidity levels and Moringa oleifera concentration on the effectiveness of coagulation in water treatment *Water Science and Technology*. 59: 1551–8. DOI: https://doi.org/10.2166/wst.2009.155.