MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Jebun *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 21 (2025) 1506-1512

RESEARCH ARTICLE

Performance of Myco-coagulant from *Lentinus squarrosulus* for Turbidity Reduction in a Settling Column

Nessa Jebun[a], Abdullah Al Mamun[b*], Md. Zahangir Alam[c], Raha Ahmad Raus[c], Radhia Nedjai[c,d]

[a]Presidency International School, Panchlaish-4203, Chattogram, Bangladesh; [b]Cataclysmic Management and Sustainable Development Research Group (CAMSDE), Department of Civil Engineering, Kulliyyah of Engineering, International Islamic University Malaysia (IIUM), Kuala Lumpur, Malaysia; [c]Department of Chemical Engineering and Sustainable Development, Kulliyyah of Engineering, International Islamic University (IIUM), Kuala Lumpur, Malaysia; [d]Department of Biology, Faculty of Science, Badji Mokhtar University (UBMA), 23000 Annaba, Algeria

Abstract Coagulation and flocculation are integral basic unit processes for conventional water treatment plants. Usually, chemical coagulants are used in most of the treatment plants. However, the search for natural bio-coagulants is ongoing to reduce the negative impacts of chemical coagulants on human health and the environment. In this research, a natural bio-coagulant from a local fungus (*Lentinus squarrosulus*) was produced. The sedimentation process in river water by this myco-coagulant was investigated using a settling column. Kaolin suspension was used as synthetic turbid water for the settling column tests. Detention time and the overflow rate of the particles are necessary to design sedimentation basins for water treatment plants. As such, tests were conducted to plot the iso-removal lines for the kaolin particles. Such data is required to design sediment or settling basins for the water treatment facilities. Therefore, detention times and overflow rates of the kaolin particles were calculated for an optimum myco-coagulant dose of 1% (v/v). To reduce 80% of the initial turbidity from the kaolin suspension, the overflow rate and detention time of the sedimentation tank should be 41.6 m/day and 59.5 minutes, respectively. In contrast, similar ranges of overflow rates and detention times could remove only about 23% of the turbidity from the kaolin suspension without any myco-coagulant. This novel, natural and biodegradable coagulant is found to have the potential for reducing turbidity in river water; therefore, also can be a good candidate for the coagulation-flocculation process in water treatment plants.

Keywords: Myco-coagulant, particle size distribution, river water treatment, sediment basin, turbidity.

***For correspondence:**
mamun@iium.edu.my

Received: 30 Sept. 2022
Accepted: 11 June 2024

Introduction

River contamination is widespread throughout much of the world [16]. In emerging nations, river pollution issues pose risks to both the environment and people's well-being [20]. The main factors in river pollution, that deter people from using the rivers, include a high amount of fine suspended solids and turbidity. The sources of sediment pollution are land-clearing and construction activities, soil erosion, deforestation, etc. [2]. Sediments play a significant role as carriers of additional inorganic and organic contaminants in the water bodies [18]. Considering this, reducing fine suspended solids from river water will considerably improve water quality and prevent the degradation of ecosystems.

For many years, people have employed traditional water treatment methods like coagulation-flocculation, sedimentation, and filtration [3]. Common chemical coagulants are typically employed to clean the water. Several chemical coagulants can be used to treat river water, including potash alum, poly aluminium chloride (PAC), and aluminium sulphate ($Al_2(SO_4)_3$) for Ganga River water [12], poly aluminium chloride for Karoon River water [8], PAC and $Al_2(SO_4)_3$ for Yellow River water [22], and ferric chloride ($FeCl_3$) and $Al_2(SO_4)_3$ for Huangpu River water [10]. However, numerous studies have demonstrated that utilising chemical coagulants can cause major health issues, including neuropathological illnesses [13] and strong environmental and human carcinogens [17]. Additionally, alum salts are frequently utilized in contemporary water treatment facilities; this produces a significant amount of sludge, with alum being primarily concentrated in the sediment sludge. In addition, it is challenging to reduce the volume of sludge using a dewatering system so that it may be disposed of safely without harming the environment [21]. The discharge of alum sludge can have negative effects on water quality, and aquatic communities may suffer long-term effects [19].

A solution to the issues would be to investigate novel microbial coagulants. Bio-coagulants are biodegradable and safe for both the environment and people's health. Therefore, it would provide new sources and knowledge to get eco-friendly, renewable, and efficient microbial coagulants that reduce the turbidity of river water. The application of bio-coagulants is now promising in river water treatment research due to their high efficiency in reducing water turbidity [16]. The efficiency of a good bio-coagulant varies between 80 to 95%. However, bio-coagulant production and their flocculation activity are the main concerns limiting the wide application of microbial coagulants. The production cost was high due to the expensive growth media of bio-coagulants [9], screening of efficient strain was hard, and the mechanism of microbial flocculation is not completely specific yet. Recently, many researchers are still exploring novel potential microbial coagulants with high flocculation activity and developing bio-coagulant yield. Nowadays, the toxicity of metabolites is one of the concerns for human health. Some filamentous fungal species produce metabolites which are mycotoxins that can easily contaminate foods and feeds. Myco coagulant produced from white rot fungi was tested using turbid synthetic water with different turbidity levels [11]. *L. squarrosulus* produced a myco-coagulant that was effective in the removal of turbidity of a kaolin suspension and river water [6]. The optimal conditions for the production of coagulant from *L. squarrosulus* were malt extract concentration 0.1% (g/l), initial pH 7.0 and inoculum dose 3% (v/v) for 6 days of cultivation. The myco-coagulant produced under this optimum condition achieved efficiency as high as 95%. The coagulant's stability indicated that it can work at different pH ranges between 4-8 and can be stored at room temperature (25 ºC) [7].

It has been reported in recently published works [5] that the novel myco-coagulant from *Lentinus squarrosulus* could be a potential replacement for chemical coagulants [8]. Therefore, this study was done to see how well the lab-scale settling column worked to get rid of turbidity from kaolin suspension and river water using a new myco-coagulant. The sedimentation process was investigated by using the myco-coagulant in a lab-scale cylindrical settling column to calculate particle settling velocity. The effect of myco-coagulant dose on the performance of settling during the column experiments was determined.

Materials and Methods

Sample Collection

River water samples were collected from three different locations of the Pusu River at IIUM Gombak campus, Malaysia. One sample was collected from each location and mixed to make a composite sample (Figure 1). The samples were stored in a closed container and kept in a chiller at 4 ºC for subsequent use.

Equipment and Instruments

During the research experiment, well-equipped laboratories were used where all equipment and machines were in good condition. The equipment employed includes an autoclave, orbital shaker, laminar air flow chamber, laboratory incubator, chiller, freeze dryer (LABCONCO), portable turbidimeter 2100Q HACH, vacuum pump, measuring balance, pH meter, cylindrical settling column, constructed lab scale channel.

Microorganism and Growth Conditions

The *Lentinus squarrosulus* was isolated from Pusu River water and sediment. A strain was maintained at room temperature (28±2 ºC). It was subcultured on 3.9% (w/v) of potato dextrose agar (PDA) plates once every month and stored in the chiller (at 4 ºC) until further usage.

(a) River water

(b) *Lentinus squarrosulus* in agar plate

Figure 1. Composite river water sample from where the fungus was extracted

Myco-Coagulant Preparation

The screening medium [0.1% malt extract (w/v)] was mixed with 1 L of distilled water to prepare the liquid broth. The initial pH of the broth was adjusted to 7.0 ± 0.1 using hydrochloric acid (HCl) or sodium hydroxide (NaOH). The medium was then inoculated with 3% (v/v) fungal mycelial inoculum after being autoclaved at 121 °C for 15 minutes to sterilize it. The liquid culture was then incubated for 6 days at 30 ± 2 ºC with 150 rpm of agitation in a rotary shaker. Supernatants were collected as the main myco-coagulant.

Settling Characteristics

Particle Size Analysis

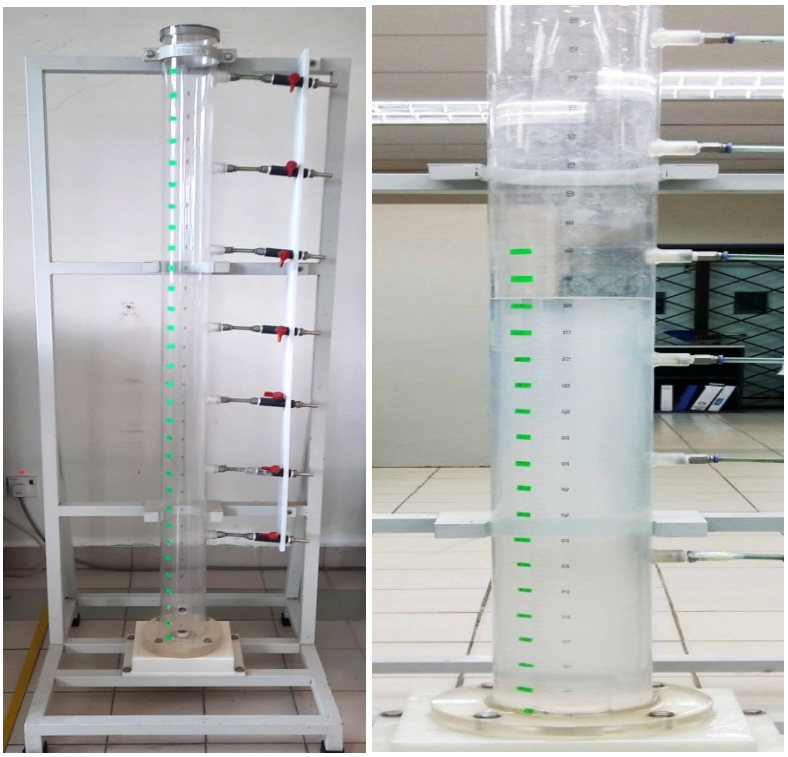
Particle size distribution was determined utilizing a Mastersizer 1000-laser diffraction particle size analyser (USA). The detected sizes ranged from 0.1 to 1 µm. The samples of control and treated kaolin suspension and river water were tested for particle size determination.

Settling Column

An experimental set-up was used with a transparent glass cylinder settling column. The height of the column was 150 cm height with a 9.5 cm diameter and a working volume of 11 L. The setup is shown in Figure 2. Initial turbidity of kaolin suspension was recorded at 600 ± 10 NTU, pH at 7.0 and river water at 500 ± 10 NTU and the pH value was recorded at 7.5 ± 0.2. Each Jar contained 0.5 L kaolin suspension and river water was added with 1% (v/v) myco-coagulant. To study the effect of the control (without any myco-coagulant), 1% (v/v) of nutrient broth was used instead of the coagulant. The mixture was then rapidly stirred at 250 rpm for 7 min and slowly stirred for 22 min, which was determined based on an optimization exercise to determine the optimum process parameters for the Jar Test to remove the maximum amount of turbidity from the kaolin water. After that, the flocculated mixture was poured into the glass cylinder settling column for experimenting.

Samples were collected from six sampling points in the column at a 5-minute interval and measured for residual turbidity by using the portable turbidimeter. The untreated (control) and treated kaolin suspension and river water were tested separately in the settling column. The turbidity removal percentage was calculated according to Equation 1.

$$Removal\ (\%) = \frac{initial\ turbidity\ - final\ turbidity}{initial\ turbidity} \quad (1)$$



(a) Empty (b) With water sample

Figure 2. Settling column used for studying settling characteristics

The overflow rate and detention time for targeted removal percentages were calculated by using the equation as follows:

$$V_0 = \frac{h}{t} \times 1440 \ \frac{min}{d} \quad (2)$$

where, V_0 is the overflow rate m/day, h is the depth of column; and t is the detention time.

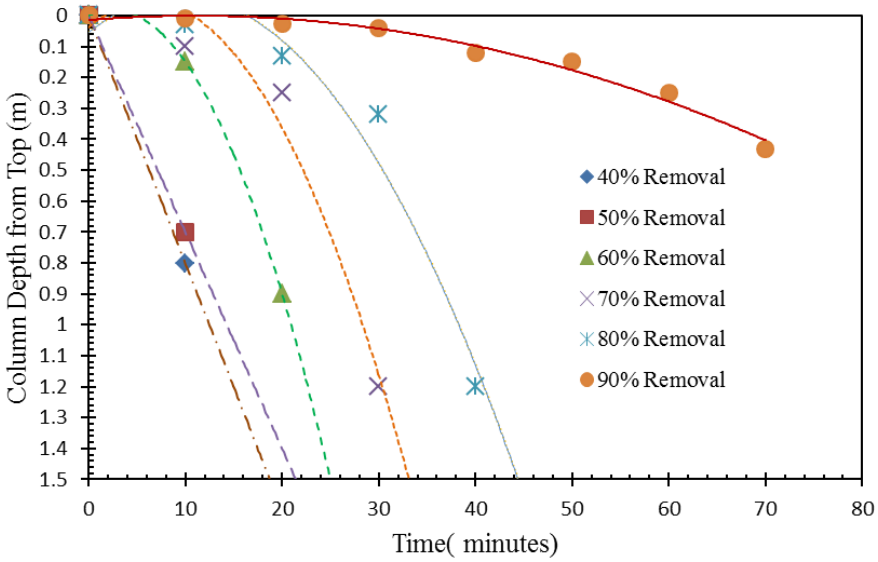
Results and Discussion

Settling Characteristics

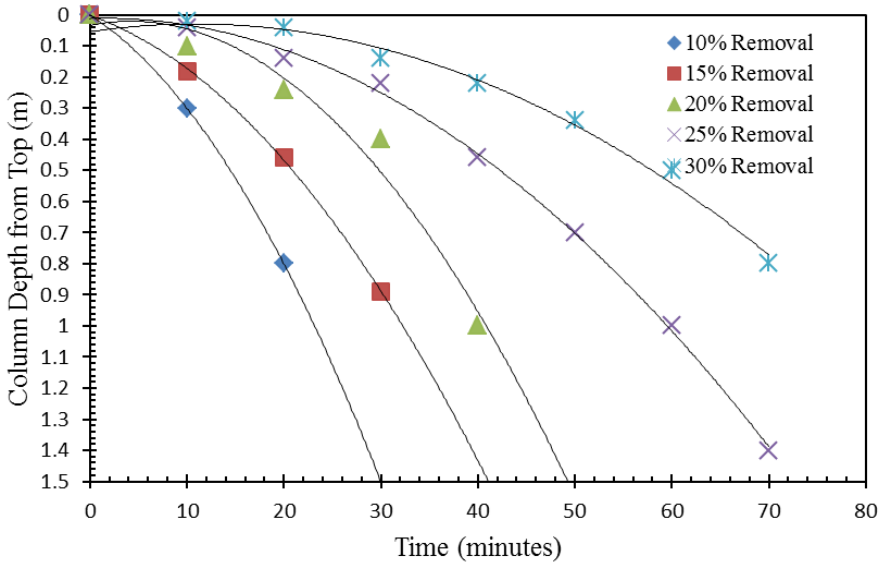
This study was carried out to evaluate the settling performance of flocs of myco-coagulant-treated kaolin suspension and river water in a cylindrical settling column. The control experiments were conducted without coagulant.

Sedimentation Parameters for Kaolin Suspension

Sedimentation is one of the integral steps after the coagulation-flocculation process. The evaluation was done in terms of turbidity reduction from kaolin suspension in the lab scale column (Figure 2). The sedimentation ability i.e. settling rate manifested as turbidity removal percentage was measured by collecting samples at various time intervals along the 150 cm height of the settling column. The same volume of treated and untreated kaolin suspensions was used in the experiments. Figure 3 shows the iso-removal line for treated and untreated kaolin suspension. The result of the scale-up factor was calculated by detention time and overflow rate of 59.5 mins and 41.6 m/day respectively for 80% turbidity removal from treated kaolin suspension. The iso-removal line for treated kaolin showed the turbidity removal rate (40 to 90%) of different depth zones (between 0 to 1.5 m) of the settling column for the correspondence time (0 to 70 mins).



(a) treated kaolin suspension



(b) untreated kaolin suspension as a control

Figure 3. Iso-removal lines (in %) for turbidity removal

The sedimentation performance was also determined for untreated kaolin suspension (control) to compare the settling ability between treated and untreated kaolin suspension. The iso-removal line for untreated kaolin suspension is shown in Figure 3 (b). For the control experiment, the scale-up factor for 28% turbidity removal was achieved by the detention time and overflow rate of 105 mins and 24.05 m/day, respectively. The results of the untreated kaolin suspension showed the highest removal of 23% turbidity at 60 minutes while treated kaolin particles were removed by 80% at the same time. The comparison results indicate that the myco-coagulant produced by *Lentinus squarrosulus* could be applied effectively to reduce the colloidal particles. No evidence could be found in the literature, regarding the sedimentation rate in a settling column test by using myco-coagulant for water treatment.

The flocculation mechanisms of bio-coagulants have been studied by several researchers. For instance, *Aspergillus flavus* produced protein and polysaccharide bio-coagulant [1] and *Aspergillus parasiticus* produced sugar and protein bio-coagulant [4] have shown good flocculation activity to

reduce turbidity for wastewater and river water treatment. In this case, the new myco-coagulant produced by *Lentinus squarrosulus* was able to entrap colloidal particles from the kaolin suspension resulting in the formation of a big floc during the coagulation-flocculation process which enhanced the sedimentation rate in the settling column. It is probably due to extracellular polymeric materials produced and released by the fungus. The myco-coagulant exhibited promising performance in settling kaolin particles in water without the addition of the cations. The new myco-coagulant can be considered a green bio-coagulant because of no generation of secondary pollution due to the presence of any chemical coagulant aid [8].

In this part of the experiments, a settling column test was carried out to evaluate the performance of the sedimentation using the new myco-coagulant. Based on the settling column test results, a typical rectangular (length 25-40 m, depth 3.5 m, width 3-24 m) or circular (diameter 12-45 m and depth 4.5 m) sedimentation basin can be used for water treatment. However, these dimensions may vary depending on the inflow of raw water into the treatment plant.

Conclusions

A novel bio-coagulant was produced from the liquid-state culture of a locally isolated fungus (*Lentinus squarrosulus*). The coagulant is named “myco-coagulant” due to its extraction from fungus. The sedimentation parameters of the coagulant in a settling column are reported in this work. Settling performance was investigated to reduce turbidity in kaolin suspension. The detention time of 59.5 mins and overflow rate of 41.6 m/day were required to reduce 80% turbidity in the kaolin-mixed turbid water. This study showed that myco-coagulants produced by *L. squarrosulus* were effective in the removal of turbidity from kaolin suspension. The settling column test data also can be used to design actual sedimentation basins. No such results, on the performance of bio-coagulant from fungus, are reported in the literature. As such, this work can be considered a pioneer in the field of the application of myco-coagulant for settling column tests. However, there is are need for further research to develop a large-scale sedimentation process by using the myco-coagulant for the reduction of turbidity in river water.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgement

The authors would like to acknowledge the IsDB Grant No MYS1009 (SPI20-001-0001) for funding this research project. Thanks are also due to the Research Management Center (RMC) for financial management and monitoring of project progress and the Faculty of Engineering of IIUM for the scientific equipment and facilities.

References

[1] Aljuboori, A. H. R., Idris, A., Abdullah, N., & Mohamad, R. (2013). Production and characterization of a bio-coagulant produced by *Aspergillus flavus. Bioresource Technology, 127*, 489–493.
[2] Chan, N. W. (2012). Managing urban rivers and water quality in Malaysia for sustainable water resources. *International Journal of Water Resources Development, 28*(2), 343–355.
[3] Davis, M., & Cornwell, D. (2013). *Introduction to environmental engineering* (5th ed.). McGraw-Hill.
[4] Deng, S., Yu, G., & Ting, Y. P. (2005). Production of a bio-coagulant by *Aspergillus parasiticus* and its application in dye removal. *Colloids and Surfaces B: Biointerfaces, 44*(4), 179–186.
[5] Jebun, N., Al-Mamun, A., Alam, M. Z., & Raus, R. A. (2018). Optimization of flocculation process for a new myco-coagulant to reduce water turbidity. In *Regional Conference on Science, Technology and Social Sciences (RCSTSS 2016)* (pp. 271–281).
[6] Jebun, N., Al-Mamun, A., Alam, M. Z., & Raus, R. A. (2016). Fungal coagulant to reduce turbidity of river water. *ARPN Journal of Engineering and Applied Sciences, 11*(6), 4094–4099.
[7] Jebun, N., Mamun, A., Alam, M. Z., & Raus, R. (2018). Production and stability of myco-coagulants from *Lentinus squarrosulus* RWF-5 and *Simplicillium obclavatum* RWF-6 for reduction of water turbidity. *IIUM Engineering Journal, 19*, 48–58.
[8] Jebun, N., Alam, M. Z., Mamun, A. A., & Ahmad Raus, R. (2022). Novel myco-coagulant produced by *Lentinus squarrosulus* for removal of water turbidity: Fungal identification and flocculant characterization. *Journal of Fungi, 8*(2), 192. https://doi.org/10.3390/jof8020192

[9] Li, Y., He, N., Guan, H., Du, G., & Chen, J. (2003). A novel polygalacturonic acid bio-coagulant REA-11 produced by *Corynebacterium glutamicum*: A proposed biosynthetic pathway and experimental confirmation. *Applied Microbiology and Biotechnology, 63*(2), 200–206.
[10] Liu, W. J., Wang, K., Li, B. Z., Yuan, H. L., & Yang, J. S. (2010). Production and characterization of an intracellular bio-coagulant by *Chryseobacterium daeguense* W6 cultured in low nutrition medium. *Bioresource Technology, 101*(3), 1044–1048.
[11] Mirzaiy, A., Takdastan, A., Alavi, N., & Mohamadian, H. (2012). Removal of turbidity, organic matter, coliform and heterotrophic bacteria by coagulants poly aluminium chloride from Karoon River water in Iran. *Asian Journal of Chemistry, 24*(6), 2389–2393.
[12] Mukherjee, S., Bhattacharya, A. K., & Mandal, S. N. (2014). Evaluation of performance of different aluminium-based coagulants and aids in river water clarification. *International Journal of Sustainable Development and Planning, 9*(3), 417–429.
[13] Muyibi, S. A., & Alfugara, A. M. S. (2003). Treatment of surface water with *Moringa oleifera* seed extract and alum: A comparative study using a pilot scale water treatment plant. *International Journal of Environmental Studies, 60*(6), 617–626.
[14] 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.
[15] Okaiyeto, K., Nwodo, U. U., Mabinya, L. V., Okoli, A. S., & Okoh, A. I. (2016). Evaluation of flocculating performance of a thermostable bio-coagulant produced by marine *Bacillus* sp. *Environmental Technology, 37*(14), 1829–1842.
[16] Patterson, J. J., Smith, C., & Bellamy, J. (2013). Understanding enabling capacities for managing the “wicked problem” of nonpoint source water pollution in catchments: A conceptual framework. *Journal of Environmental Management, 128*, 441–452.
[17] Rudén, C. (2004). Acrylamide and cancer risk: Expert risk assessments and the public debate. *Food and Chemical Toxicology, 42*(3), 335–349.
[18] Sekabira, K., Origa, H. O., Basamba, T. A., Mutumba, G., & Kakudidi, E. (2010). Assessment of heavy metal pollution in the urban stream sediments and its tributaries. *International Journal of Environmental Science and Technology, 7*(3), 435–446.
[19] Sotero-Santos, R. B., Rocha, O., & Povinelli, J. (2005). Evaluation of water treatment sludges toxicity using the Daphnia bioassay. *Water Research, 39*(16), 3909–3917.
[20] Su, S., Li, D., Zhang, Q., Xiao, R., Huang, F., & Wu, J. (2011). Temporal trend and source apportionment of water pollution in different functional zones of Qiantang River, China. *Water Research, 45*(4), 1781–1795.
[21] Wu, C. H., Lin, C. F., & Chen, W. R. (2004). Regeneration and reuse of water treatment plant sludge: Adsorbent for cations. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering, 39*(3), 717–728.
[22] Yang, Z., Gao, B., & Yue, Q. (2010). Coagulation performance and residual aluminum speciation of Al2(SO4)3 and polyaluminum chloride (PAC) in Yellow River water treatment. *Chemical Engineering Journal, 165*(1), 122–132.