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FUNGAL FLOCCULANTS TO REDUCE TURBIDITY OF RIVER WATER

N. Jebun, Abdullah Al-Mamun, Md. Zahangir Alam and Raha binti Ahmad Raus
Bioenvironmental Engineering Research Centre, Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, Jalan Gombak, Kuala Lumpur, Malaysia
E-Mail: mamun@iium.edu.my

ABSTRACT

River water contains both dissolved and suspended particles. Coagulation and flocculation processes are used to separate the suspended solids from the water. Conventionally, suspended solids and turbidity are removed from raw water by various chemical coagulants but most of them are costly and non-ecofriendly. Whereas, the bioflocculants are environment-friendly and could be used as coagulants. In general, bioflocculants cause aggregation of particles and cells by bridging and charge neutralizing. Most of the solids suspended in water possess negative charges and repel each other, for which they do not settle in the water body. This present study attempted to reduce turbidity of river water and kaolin suspension using fungal coagulants in Jar apparatus. Bioflocculant producing filamentous fungi were isolated from river water. Six strains showed good flocculating performance. Among them, supernatant of RWF-05 and RWF-06 showed turbidity removal of 95% and 75% from kaolin suspension, respectively. However, the removal of turbidity from river water was rather low with 23% and 22%, respectively. Dried biomass of RWF-03 showed a good flocculating rate of 80% after 24h settling time to kaolin suspension. The results showed that the clay particles of river water and kaolin were flocculated by the fungal supernatant and dried biomass and, as a result, they reduced the turbidity of river water.

Keywords: coagulation, kaolin, flocculation, fungal strain, river water, turbidity.

INTRODUCTION

Bioflocculant is a kind of biodegradable macromolecular product secreted by certain microorganisms. Their main components are the glycoprotein, polysaccharide, protein, etc [2, 18]. Due to their merits of high efficiency, security, nontoxic biodegradation and no secondary pollution, bioflocculants have great potentials for industrial application and have become a research focus in water treatment [15]. Recently, a few filamentous fungi have attracted high biotechnological attention to treat water and wastewater due to their entrapment capability [6, 3]. For its avirulence, bioflocculants are widely used for the recovery of suspended solids (SS). In contrast, chemical flocculants cannot be easily degraded in nature and may result in some health and environmental problems [11]. Inorganic and organic synthetic flocculants are commonly used in water and wastewater industries [19]. However, these chemicals may have detrimental effects on health and the environment [4].

Various conventional methods are used for purification of water and removing the pollutant contaminants, but most of them are costly and non-ecofriendly [9]. Many water treatment processes have been developed and used for decades, such as coagulation–flocculation units, sedimentation basins, sand filtration, and disinfection units [10]. Studying the flocculation mechanism could help us to well understand the role of bioflocculants in water and wastewater treatment and to improve the actual good treatment mechanism. In general, bioflocculants cause aggregation of particles and cells by bridging and neutralizing charges [15]. Polymer bridging proposes that cation-mediated bridges between the kaolin particles and bioflocculant chains primarily form flocs [12, 16].

The aim of this study was to investigate the turbidity removal potentiality of six filamentous fungi isolated from river water and to determine the flocculation activity in terms of reducing turbidity (by Jar test) from both river water and kaolin suspension.

MATERIALS AND METHODS

Culture media

Potato Dextrose Agar (PDA) was used in this study and it was prepared according to the manufacturer's instructions, 39 g of PDA was dissolved in 1000 ml of distilled water and then sterilized (autoclaved) at 121ºC and pressure of 15 Pa for 15 minutes. Initial pH was adjusted to 5.8±2.Then incubated at 32±2 ^{0}C for 5-7 days. Subcultures have conducted once in a month and stored at 4 ^{0}C for further use.

Isolation of fungi

Water samples were collected from the *Pusu* River at IIUM campus. The fungi were isolated from river water using the spread plate technique [14]. One ml raw river water sample was dissolved in 9 ml sterilized distilled water. The river water suspension was diluted up to 10^{-1}-10^{-5}. The isolated samples were inoculated on prepared PDA plates. The inoculated plates were incubated at ambient temperature (30 ± 2 ^{0}C) for 5-7 days. Colony formation was observed after the incubation period. The young fungal colonies were aseptically picked up and transferred to fresh sterile PDA plates to obtain pure cultures. The pure cultures on PDA plates were

grown at 30 ± 2 ^{0}C for 5-7 days and stored at 4^0 C until required for future use.

Inoculum preparation

The inoculum of spore suspensions was used for spore producing strains while mycelial suspensions were used for basidiomycete group.

Spore suspension

Five-day-old culture plates were transferred into Erlenmeyer flask (250 ml) containing 100 ml of sterile distilled water. It was shaken in a rotary shaker with 150 rpm for 24 h. The suspended solids were filtered by Whatman filter paper no. 1 and the filtrate were used as inoculum after measuring its strength (1-2.5 x 10^8 spore/ml) by Hemocytometer. All flasks, funnels, filter papers and distil water were sterilized before use.

Mycelial suspension

Seven-day-old cultures grown on PDA plates were used for mycelial suspension. The mycelia in plates were washed successively three times with 100 ml of sterile distil water by a glass rod and poured into 100 ml of the flask to use as final inoculum after measuring its dry mycelial strength (340 mg/L).

Bioflocculant production

The fungus for fermentation was grown in 250 ml Erlenmeyer flasks with medium containing 0.5% malt extract and mixed with 1litter distilled water. The media sterilized by autoclaving it at 121 °C for 15minutes.Then inoculated 2% (v/v) fungal inoculum into liquid media. Liquid culture incubated in a rotary shaker with 150rpm at room temperature for 7 days. Initial pH of the culture was adjusted at 7.00 ± 0.1 using 1M NaOH or 2M HCl. After 7 days of cultivation time, all fungus was harvested and separated their supernatant and biomass by Whatman No.1 filter paper. The supernatants were stored at 4 ^{0}C for further use.

Dry weight of biomass

The dry weight of the filamentous fungi was measured by drying at 70-80 ^{0}C [5]. The biomass samples were filtered and then dried at 70-80 ^{0}C in an oven for few hours until become fully dried then taken the total dry weight of biomass.

Evaluation of flocculating rate by jar test

Kaolin suspension and river water obtained from Pusu River, IIUM, Malaysia, were used to determine the flocculating rate of the bioflocculant to reduce turbidity. A 0.7 g/L kaolin clay (R and M chemicals, UK) was mixed in 1 L of water. Initial turbidity was recorded at 900 ±10 NTU and pH at 7.00 ± 0.1. Each Jar contained 500 mL kaolin suspension and river water were added 1.2% supernatant (v/v) and dried biomass 0.2 g/l then the jar set up operated at 120 rpm speed with three different mixing time (20, 30 and 40 minutes) and allowed to settled for 30 minutes. Then the top layer of water in each Jar was collected with a micro pipette and flocculation efficiency was calculated based on % reduce in turbidity. All the experiments were conducted in triplicate. For the control experiment, Kaolin suspension and river water without added supernatant were used as control. The turbidity removal was calculated as follows:

$$\text{Turbidity removal efficiency (\%)} = (A-B)/A \times 100 \quad (1)$$

Where A is the initial turbidity value and B is the turbidity value after flocculation.

Measurement of turbidity and pH

Initial and final turbidity were measured by using Nephelometric turbidity unit (NTU) (standard method 2130 B) with a portable turbidimeter 2100Q HACH, USA [3]. The pH value of water was measured by a pH meter (Sartorius PB-10, Germany).

RESULTS AND DISCUSSIONS

Screening for potential coagulant producing fungi

Turbidity removal from kaolin suspension

Cultures of six filamentous fungi in malt extract, isolated from river water were Jar tested using fungal supernatants for the ability to flocculate Kaolin clay suspension and river water and initial turbidity was recorded 900 ± 10 NTU (Figure-1). The results are summarized in Table-1. Table-1 shows that six fungal supernatant showed good turbidity removal (%) in Kaolin suspension. The result showed that RWF-5 and RWF-6 could effectively use to reduce the clay particles from water. The turbidity removal was significantly decreased from 900 NTU to 46 NTU (95%) by the biocoagulant of RWF-5 and 200 NTU by the RWF-6. [13] Reported that *Rhizopus sp.* M9 and M17 were showed good turbidity removal rates at 54% and 92% of the potato starch wastewater respectively. Similar results were obtained for *Aspergillus flavus* due to the presence of hydroxyl, amide, carboxyl and methoxyl groups which are suitable functional groups for the bioflocculation process [2].

Figure-1. Jar test using fungal coagulants.

Effects of mixing time

Mixing during flocculation process provides close encounters between particles and flocculating agents [7]. In order to achieve an optimum flocculating rate, the effect of high and low mixing time on the flocculating rate of six fungi were investigated. As shown in Figure-2, the flocculating rate at a mixing time of 20 mins was slightly higher than 40 mins with a mixing speed at 120rpm in RWF-1 and 30mins was slightly higher than 40mins in RWF-3. At high mixing times, the flocculating rate increased from 91% to 95% in RWF-5 and 71% to 77% in RWF-6. Moreover, the current study found that the flocculating rate at mixing time of 40 minutes was higher than those at 20 mins and 30 mins, within mixing speed at 120 rpm. These findings show that high mixing time encourages more kaolin particles to aggregate than the other low speeds of 20mins and 30mins except RWF-1 and RWF-3.

Table-1. Turbidity reduction by fungal coagulant.

Fungus strain	Final turbidity (NTU) (Control)	Final turbidity (NTU) Treatment)
RWF-1	520	394
	580	600
	560	440
RWF-2	520	500
	580	558
	560	460
RWF-3	600	570
	630	450
	670	510
RWF-4	600	560
	630	600
	670	510
RWF-5	580	75
	603	57
	571	46
RWF-6	580	240
	603	220
	571	200

The maximum flocculating rate observed at 40mins was 95%. Figure-3 shows dry biomass of RWF-3 entrapped the colloid particles from kaolin suspension to reduce turbidity in different settling time. Table-2 shows six fungal biomass shows turbidity removal (%) from kaolin suspension after 30 mins settling time. [8] Reported that the cation-independent polysaccharide bioflocculant produced by *B. mucilaginosus* was able to flocculate kaolin suspensions without cations.

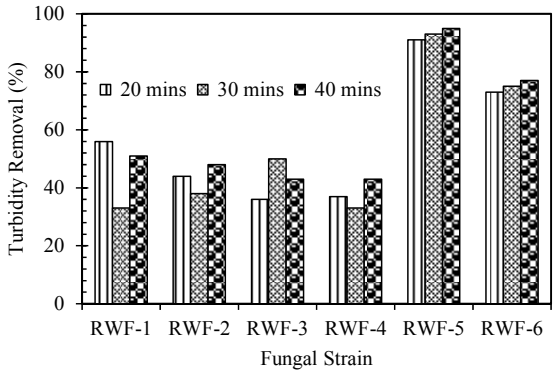


Figure-2. Effects of mixing time on turbidity removal.

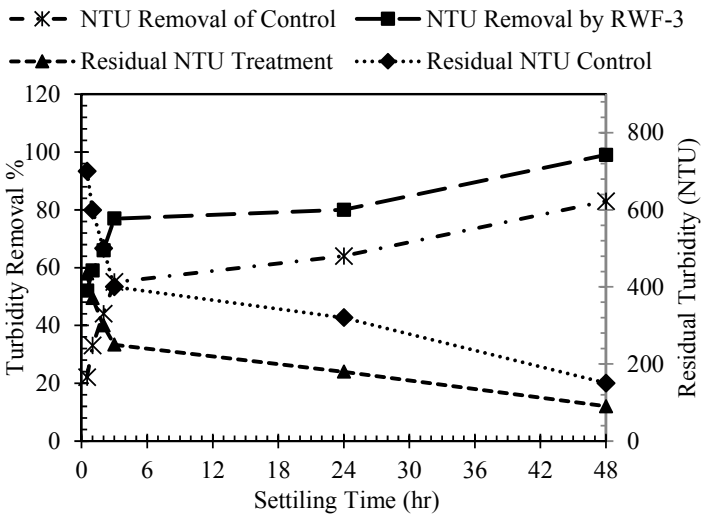


Figure-3. Settling time on flocculating activity of RWF-3 dry biomass.

Table-2. Turbidity removal (%) by fungal dry biomass from kaolin suspension.

Fungal strain	Mixing time (min)	Control		Treatment	
		Final turbidity NTU	Turbidity removal (%)	Final turbidity NTU	Turbidity removal (%)
RWF-1	20	655	27.22	525	41.66
	30	733	18.55	688	23.55
	40	687	23.66	538	40.22
RWF-2	20	655	27.22	492	45.33
	30	733	18.55	614	31.77
	40	687	23.66	617	31.44
RWF-3	20	585	35	412	54.22
	30	655	27.22	319	64.5
	40	543	39.67	369	59
RWF-4	20	585	35	412	54.22
	30	655	27.22	424	52.89
	40	543	39.67	449	50.11
RWF-5	20	585	35	477	47
	30	655	27.22	341	62.11
	40	543	39.67	390	56.67
RWF-6	20	585	35	451	49.89
	30	655	27.22	465	48.33
	40	543	39.67	498	44.67

Purification of river water

River water with initial turbidity of 900±10 NTU and pH was 7.00±0.1 was used to investigate the six fungal supernatant and dried biomass on turbidity removal %. Among them, RWF-5 and RWF-6 showed good turbidity removal (Figure-4). Figure-5 shows entrapped solid particles from river water by RWF-5. [3] Reported that bioflocculant PM-5 produced from *Aspergillus niger* and it showed high potential to treat colloids from river water at 63% of turbidity removal.

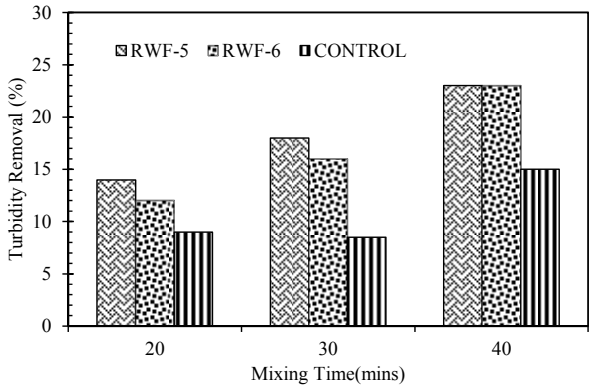
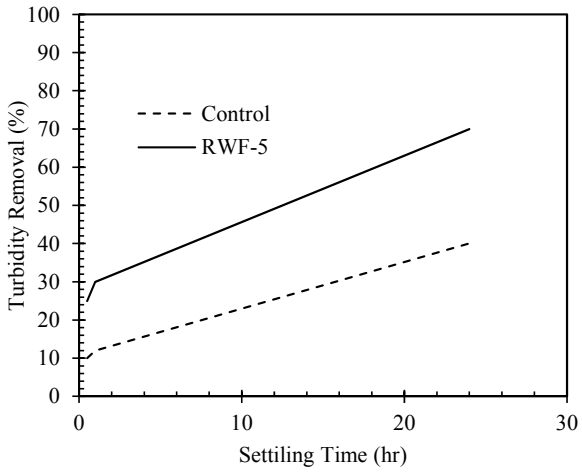
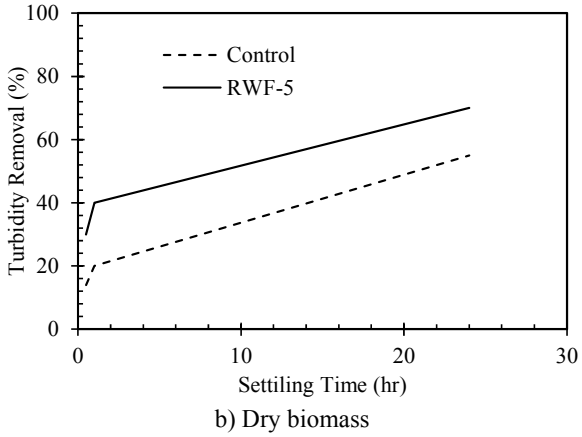


Figure-4. Turbidity removal (%) of RWF-5 and RWF-6 from river water.



a) Supernatant

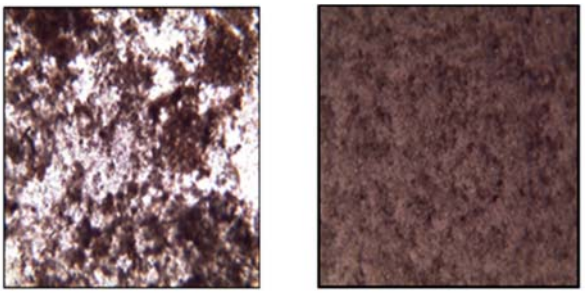


b) Dry biomass

Figure-5. Turbidity removal from river water of RWF-5.

Surface structure of RWF-3 and flocs

Optical microscopic images showed the morphology of bioflocculant RWF-3 and its flocculation to kaolin particles (Figure-6). The image in Figure-6 (a) showed precipitated kaolin particles flocculated around the RWF-3 dry biomass molecule (floc) and (b) shows the structure of kaolin particles before the flocculation. This may be due to charge neutralization mechanism [1]. Xia *et al.*, [17] reported that SEM image of TJF1 bioflocculant produced by *Proteus mirabilis* shows good attachment with kaolin clay also polymer bridging flocculation mechanism.



a) Flocculated kaolin b) Kaolin in control

Figure-6. Optical microscopic images.

CONCLUSIONS

Several fungi were tested and six fungi were able to reduce turbidity from kaolin clay suspension. Among them, RWF-5 and RWF-6 were showed good removal rate from kaolin clay suspension at 95% and 75% in Jar test after 30mins settling time respectively. RWF-5 and RWF-6 were also showed good turbidity removal from river water. RWF-3 dry biomass showed 80% removal turbidity after the 24h settling time. The potential strains were screened on the basis of showing their best results of the turbidity removal (%) from Pusu river water and kaolin clay suspension to evaluate the flocculation properties. Consideration their flocculating activity and harmlessness toward the environment some potential fungus are expected to be a good replacement of conventional synthetic flocculants and applied in water treatment.

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