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EVALUATION OF ENTRAPMENT POTENTIALITY AND TURBIDITY REMOVAL EFFICIENCY OF FUNGI

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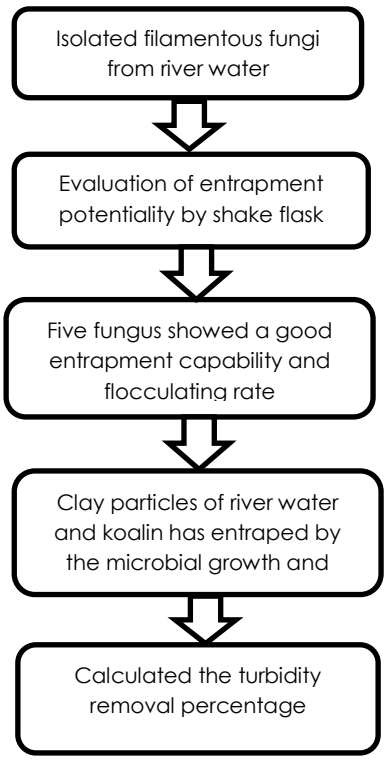
Graphical abstract



Abstract

Turbidity and suspended solids concentration promotes a number of negative effects on freshwater ecosystems. 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. Extracellular polymeric substances (EPS) produced by microorganisms play a definite role to reduce the turbidity of river water which can enhance the aesthetics of river water and other water uses. In this study, pellets /flocs have been observed of five filamentous fungi isolated from Pusu river water. The strains RWF-01, RWF-02, RWF-03, RWF-04 and RWF-05 showed a good entrapment capability and flocculating rate of 97.56%, 99.42%, 99.18%, 59.34% and 85.21% to kaolin suspension and 44.54%, 99.27%, 98.59%, 28.57% & 68.43% to river water respectively at 48h of culture time. The result showed the clay particles of river water and kaolin has entrapped by the microbial growth and, as a result, they reduced the turbidity of river water.

Keywords: River water, Kaolin clay, Turbidity, Filamentous Fungi, Flocculation

1.0 INTRODUCTION

Bioflocculants have received considerable scientific and biotechnological attention in recent years due to their biodegradability, benign nature and lack of secondary pollution of their degradative intermediates [1, 2]. In recent years, many bioflocculant-producing microorganisms have been reported including bacteria, fungi, yeast and actinomycetes [3, 4]. Studying the flocculation mechanism could help us to well understand the role of bioflocculants in water and wastewater treatment and to improve the actual treating effects. In general, bioflocculants cause

aggregation of particles and cells by bridging and charge neutralization [2]. In wastewater treatment, bioflocculants have been used to treat dye solutions [5], inorganic solid suspensions [6, 7], downstream processing, food and wastewater [2, 8] heavy metals [9] among others.

Conventional methods are in practice for purification of water and removing the pollutant contaminants, but most of them are costly and non-ecofriendly [10]. Many water treatment processes have been developed and used for decades, such as coagulation–flocculation units, sedimentation basins, slow sand filtration, rapid sand filtration and disinfection units [11]. Inorganic and organic synthetic flocculants are commonly used in water and wastewater industries [12]. However, these chemicals may have detrimental effects on heath and the environment [13]. Recently, some filamentous fungi have attracted high biotechnological attention to treat water and wastewater due to their entrapment capability [3, 14]. The main bioflocculation mechanisms are achieved through polymer bridging and charge neutralization. Polymer bridging proposes that cation-mediated bridges between the kaolin particles and bioflocculant chains primarily form flocs [15, 16].

Therefore, the aim of this study is to investigate the entrapment potentiality of five filamentous fungi isolated from Pusu river water sample and to determine the flocculation activity in terms of reducing turbidity, pH and biomass production by shake flask method in both river water and kaolin suspension.

2.0 EXPERIMENTAL

2.1 Preparation of Media

Potato Dextrose Agar (PDA) was used in this study and it was prepared according to the manufacturer's instructions thus, 39g of PDA was dissolved in 1000ml of distil water and then sterilized (autoclaved) at 121°C and pressure of 15Pa for 15 minutes. Initial pH was adjusted to 5.8±2. PDA was used for the isolation and maintenance of pure cultures of fungi.

2.2 Isolation of Fungus

River water samples were collected from the Pusu River at IIUM campus. The fungi were isolated from river water using the spread plate technique [17]. One ml raw river water sample was dissolved in 9 ml sterilized distilled water. The river water suspension was diluted up to 10^{-1}to10^{-5}. The isolation samples were inoculated on prepared PDA plates. The inoculated plates were incubated at ambient temperature (30 ± 2^0C) for 5-7 days. Colony developments were observed after an 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 ± 2^0C for 7 days and stored in the refrigerator at 4^0 C until required for further use.

2.3 Inoculum Preparation

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

Spore suspension
5 days old cultures plates were transferred into Erlenmeyer flask (250ml) containing 100ml of sterile distilled water. It was shaken in a rotary shaker with 150rpm for 24h.The suspended was filtered by Whatman filter paper no. 1 and this filtrate was used as inoculum after measuring its strength (1-2.5 x 10^8spore/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 100ml of sterile distil water by a glass rod and poured into 100ml of the flask for use as final inoculum after measuring its dry mycelial strength (340mg/L).

2.4 Evaluation of Entrapment Potentiality

Kaolin suspension and river water were used to determine the flocculating rate of the bioflocculant to reduce turbidity. A 0.5 - 0.7g/L kaolin clay was suspended in 1 L of tap water. Initial turbidity was recorded after added 0.5% malt extract with kaolin suspension and Pusu river water at the turbidity of 800±100NTU. The experiment was conducted in 250 ml Erlenmeyer flask containing 100ml kaolin suspension and Pusu river water and incorporated with 0.5% malt extract then autoclaved at 121^0C for 15 minutes. Then inoculated 2% (v/v) fungal spore suspension (1-2.5X10^8spore/ml) and mycelial suspension. Liquid culture incubated in a rotary shaker with 150rpm at 32± 2^0C for 7 days observations. Initial pH of the culture was adjusted at 7.00 using 1M NaOH or 2M HCl. Sample was taken at 24h time intervals to determine final turbidity, final pH & biomass weight. By measuring the decrease in turbidity of the upper phase, the degree of flocculation was measured with a turbidimeter. In the control experiment, culture medium without inoculated was used as a control. The turbidity removal was calculated as follows:

Turbidity removal efficiency (%) = (A-B)/A x 100 (1)

where, A is the initial turbidity value and B is the turbidity value after treatment.

2.5 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).

2.6 Dry weight of Biomass

The dry weight of the filamentous fungus was measured by drying at 70-80^{0}C [18].The biomass samples were filtered and dried at 70-80^{0}C in an oven for 2 hours then taken the total dry weight of biomass.

3.0 RESULTS AND DISCUSSION

3.1 Effect of Culture Time on Turbidity Removal

Figure 1 shows removal rate of five filamentous fungi from kaolin and river water in 48h culture time. The rate of removal turbidity period was faster in kaolin then river water. Overall, the removal percentage was up to 90% within 6 days culture time except RWF-4 in river water. The flocculating rate increased after 24h and reached a maximum value of 99% of RWF-2, RWF-3 and other three (RWF-1, RWF-4 & RWF-5) raised gradually within 3-7 days and decreased thereafter. This may be due to enzymatic activity and cell lysis [19]. The RWF-2 and RWF-3 were immobilized on solid particles by the growth of flocs within 72 hours cultivation time and they showed 90% removal rate of turbidity from river water and kaolin suspension (Table 1).

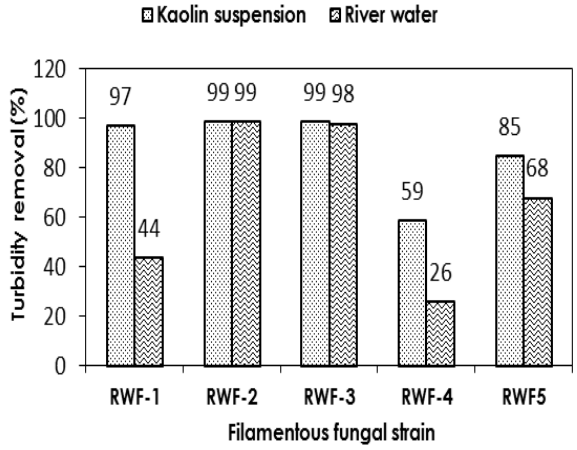


Figure 1 Entrapment rate (%) of after 48h culture time of five filamentous fungi

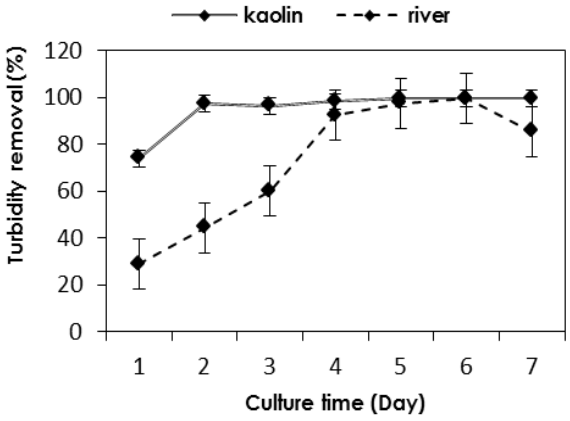
Table-1 Entrapment rate (%) in 24h, 48h and 72 h cultivation time

Fungal strain	Entrapment rate,%					
	24h		48h		72h	
	kaolin	River	Kaolin	River	Kaolin	River
RWF-1	74	28.73	97.42	44.54	96.38	60.04
RWF-2	92.05	87.12	99.4	99.27	99.61	99.46
RWF-3	99.43	97.53	99.18	98.59	97.21	99.4
RWF-4	50.94	20.0	59.34	26.0	85.16	40.96
RWF-5	20	18.46	84.47	68.43	98.83	89.77

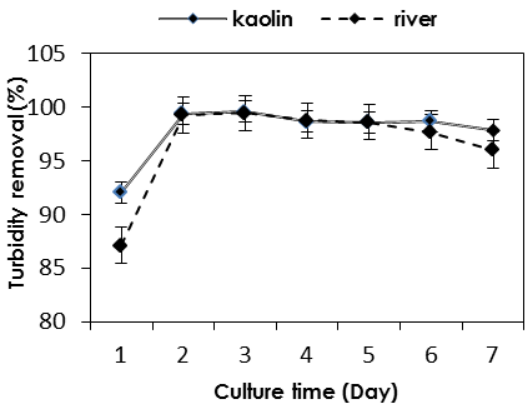
3.2 Screening for Potential Filamentous Fungi

Cultures of five filamentous fungi, which were isolated from river water and tested for the removal capacity to flocculate kaolin clay and Pusu river water.

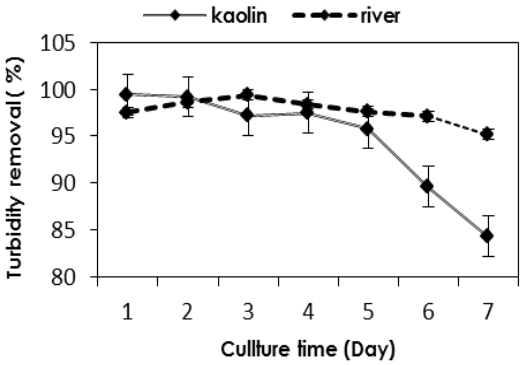
Initial turbidity was recorded at 900±50 NTU in kaolin suspension and 700±50 NTU in Pusu river water. Figure 2 shows the flocculation rate of colloid particles of river water and kaolin clay by five filamentous fungi. In this study, RWF-2 and RWF-3 were showed better entrapment potentiality and flocculating rate over 95% in river water and kaolin suspension within 48h cultivation time. This study found the flocculating rate above 90% in all fungus within 5days in kaolin suspension but 7 days in river water except RWF-4, it shows 87% on 7 days of fungal growth.



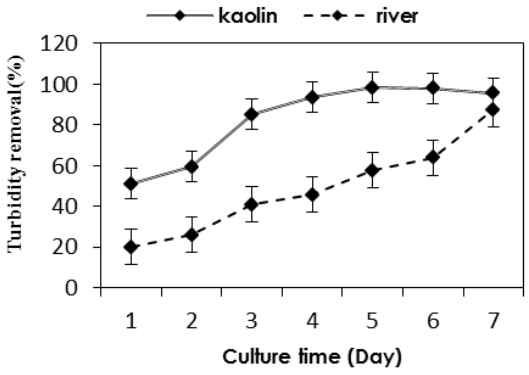
(a) Strain RWF-1



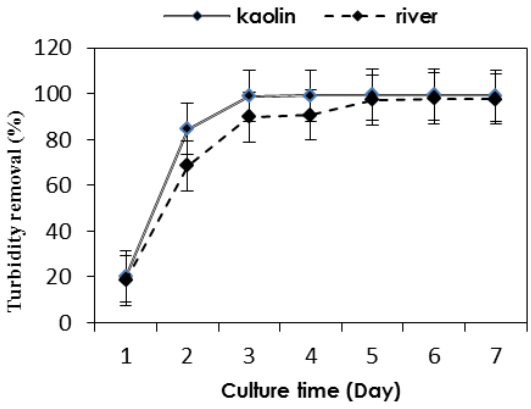
(b) Strain RMF-2



(c) Strain RWF-3



(d) Strain RWF-4



(e) Strain RWF-5

Figure 2 Turbidity removal (%) of five filamentous fungi

3.4 Formation of Pellets, Flocs and Filaments

Pellets /flocs, has been observed of five filamentous fungi (RWF-1, RWF-2, RWF-3, RWF-4 & RWF-5).The Result showed that clay particles of kaolin and river water have been entrapped by the microbial growth and, as a result they reduced the turbidity of river water (Figure 3). Bioflocs or beads formed by fungal biomass trapped the suspended solids, creating effective bioflocs and leaving a clean supernatant [20, 21, 22] have studied that the fungal growth immobilized in solid particles of wastewater sludge which has been transformed into mycelial balls and enhanced the separation process.

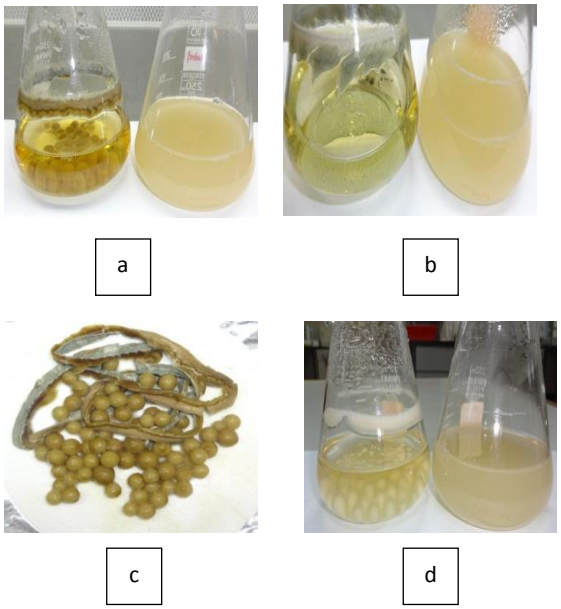


Figure 3 Images of potential microbial coagulants a) RWF-1 culture and control b) RWF-1 biomass c) RWF-3 culture and control d) RWF-5 culture and control

3.5 Determination of Biomass and pH

Table 2 shows total dry biomass (g/L) and pH from kaolin suspension in the five filamentous fungi. The growth of five filamentous fungi was studied and showed that highest biomass growth and removal turbidity rate were high in the same day in all fungus except RWF-3 which produced the highest biomass at 6 days. RWF-1, RWF-4, and RWF-5 showed high biomass yield at 5day culture time. The dry biomass growth was gradually increased then slowly decreased within 7 days harvesting period and maximum biomass growth of 2.8g/L in RWF-1. RWF-1, RWF-2, RWF-3, RWF-4, and RWF-5 were showed low pH and highest removal rate in the same culture period. The highest pH value was recorded in RWF-4 and RWF-5 (pH 5.56) at 7day and lowest pH value was recorded in RWF-2 (pH 1.7) at 72h cultivation time. Similar observations have been found by several authors with different microbes [3, 15] and [23].The maximum biomass production were achieved at the logarithmic growth phase (5-6 days) except RWF-2 where its maximum biomass was recorded after 3 days.

Table 3 showed the dry biomass g/L and pH values of five fungi in river water. RWF-1 showed highest biomass production on 6days at 2.6g/L and pH at 3.36 was low in 7 days. It might be due to the presence of organic acid components of the bioflocculant [24,14].Highest pH were recorded in RWF-3 and RWF-4 on 7day which has similar with kaolin suspension.

Table 2 Determination of biomass and pH from kaolin clay suspension

Day	Biomass g/L					pH				
	RWF1	RWF2	RWF3	RWF4	RWF5	RWF1	RWF2	RWF3	RWF4	RWF5
1	0.69	0.96	0.66	1.07	0.4	5.03	4.83	4.88	6.86	5.54
2	1.55	1.84	1.22	1.66	0.58	5.85	2.26	4.94	4.11	5.22
3	1.87	2.26	1.22	1.51	0.82	5.74	1.76	4.98	3.54	4.94
4	1.97	2.13	1.23	1.69	1.38	5.38	1.85	5.27	3.51	4.68
5	2.84	2.15	1.12	1.81	2.10	4	2.28	5.4	3.6	4.86
6	2.52	2.10	1.80	1.79	1.88	3.79	2.5	5.48	4	4.78
7	1.99	1.75	1.65	1.76	1.58	3.68	2.44	5.66	5.56	4.87

Table 3 Determination of biomass and pH from river water

Day	Biomass g/L					pH				
	RWF1	RWF2	RWF3	RWF4	RWF5	RWF1	RWF2	RWF3	RWF4	RWF5
1	0.38	0.99	0.90	0.98	0.45	6.04	5.46	4.98	4	5.6
2	1.23	1.88	1.53	1.41	0.60	4.6	2.1	4.22	4.03	4.94
3	1.34	2.25	1.52	1.60	1.00	4.57	1.99	4.00	4.48	4.78
4	1.87	2.07	1.51	1.66	1.26	4	2.32	5.53	4.1	4.71
5	2.34	2.07	1.81	1.86	1.75	3.64	2.61	5.66	5	4.71
6	2.61	1.73	1.76	2.09	2.14	3.56	2.46	5.93	6.44	4.87
7	1.99	1.63	1.47	1.81	2.38	3.56	2.51	6.04	6.65	5.05

4.0 CONCLUSION

It was observed that five filamentous fungi were able to reduce turbidity from river water and kaolin clay suspension. Among them, RWF-2 and RWF-3 were showed good removal rate at 92% and 99% from kaolin suspension after 24h culture time respectively. The potential strains were screened on the basis of showing their best results of the growth formation (pellets, flocs, and filaments), production of biomass, turbidity removal (%) and pH. During the growth of fungi, they entrapped the colloid particles from Pusu river water and kaolin clay suspension to evaluate the flocculation properties.

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