Original research papers

Characterization of Fungal Bioflocculants and its Application in Water Treatment

Abstract:

Bioflocculants of microbial origin have the advantage of being safe, biodegradable and harmless to the environment. Production of bioflocculant by two fungi isolates and the factors affecting its production were investigated in this study. Primary screening of fungi for the production of bioflocculants, efficiencies and conditions for the optimum production of the bioflocculants were determined using standard microbiological and chemical methods. *Aspergillus flavus* MCB 271 and *Aspergillus niger* MCBF 08 were the best bioflocculant producers among the fourteen fungal isolates screened. *Aspergillus flavus* optimally produced bioflocculant with glucose and peptone as sole carbon and nitrogen sources respectively. Calcium ions (Ca^{2+}) at 78.4 % served as best cation sources for bioflocculant production with optimal pH of 7 and temperature of 40^{o}C. *Aspergillus niger* MCBF 08 produced bioflocculant optimally when the media had peptone as a nitrogen source and maltose as a sole carbon source. The two species achieved maximum flocculating activity of 97% (*A. flavus* MCBF 271) and 86% (*A. niger* MCBF 08) at pH values of 7 on the 3rd day of the study and caused reduction in bacterial load of the wastewater samples by 58.73% and 60.85 % respectively. These bioflocculants are thus potential replacement for synthetic flocculants conventionally used for wastewater treatment.

Keywords: Bioflocculants, carbon sources, nitrogen sources, time course, wastewater

1.0. 1.0. Introduction

Bioflocculants are polymers, mostly, of microbial origin which floc out suspended particles from liquid medium (Xia *et al*., 2008; Ugbenyen *et al*., 2012; Nwodo *et al*., 2012). The efficiency of flocculation activities depends on the characteristics of the flocculants. In comparison with conventionally used flocculants, bioflocculants have the advantage of being safe (no toxic effects known), biodegradable and harmless to the environment. Hence, it has been topical as an alternative to conventional flocculants (Salehizadeh and Shojaosadati, 2001; Nwodo *et al*., 2013; Zaki *et al.,* 2013).

Water pollution is one of the most challenging environmental issues and has become a factor in determining the quality of life (Prasertsan *et al*., 2006). Water is a source of life and energy, even though millions of people worldwide are suffering with the paucity of safe water for drinking purposes (Bhatnagara and Sillanpaa, 2010). The global drift to the urban areas of the world, modern agricultural practices and industrialisation have contributed majorly to the high rate of pollution (Prasertsan *et al*., 2006; Li *et al*., 2013). The presence of pollutants from these sectors has made water bodies life-threatening to aquatic organisms as well as unsuitable for domestic usage (Yang *et al*., 2012).

Coagulation/flocculation is one of the most frequently used and cheapest methods in water treatment (Low *et al*., 2011; Ong *et al.*, 2012). Flocculation however, is an age long and preferred process of water purification and treatment due to its cost effectiveness, efficiency and less labour demanding (Li *et al*., 2013). Deng *et al*. (2003) observed that flocculation is an effective technique that is commonly used in wastewater treatment for removing not only suspended solids but also metal ions. Shih and Van (2001) found that flocculation could be used as an alternative to centrifugation and filtration for the separation of microbial cells from broth in food, beverage and pharmaceutical industries. Bioflocculants are needed to replace fossil-fuel based flocculants such as Polyacrylamide and poly(ethylene oxide) for industrial applications (Lee and Chang, 2018). Several research studies are searching for

effective strains that can be cultivated in low cost medium with maximum bioflocculant production rates (Kothari *et al*., 2017; Mu *et al*., 2018). The objectives of research were to determine the ability of *A. niger* and *A. flavus* to produce bioflocculants and the conditions that will enhance its optimum production by the test fungi. Also, the ability of the bioflocculants to reduction the bacterial burden of the waste water was also investigated.

2.0. Methodology

2.1 Source of Fungal Isolate

Fourteen fungal isolates were collected from the laboratory of the Department of Microbiology, Ekiti State University, Ado-Ekiti, Ekiti State. The fungi were, initially collected from the waste water, were sub-cultured from an old culture and incubated at room temperature for 7 days prior to screening for bioflocculant activity.

2.2 Screening of the Fungal Isolates for Bioflocculant Production

The four fungal isolates were screened using Bioflocculant Production Broth (BPB) adopting the method described by Didar and Ferdosi-Makan (2016). The growth medium for bioflocculant production was composed of glucose (10 g), KH_2PO_4 (2 g), $MgSO_4.7H_2O$ (0.2 g), NaCl (0.1 g), $CaCO_3$ (0.5 g) and yeast extract (0.5 g) per litre and the pH was adjusted to 6.5. The medium was autoclaved at 121– 124°C for 15 minutes. They were inoculated into McCartney bottles containing 15 mL of BPB each and incubated on a rotary shaker at 120 rpm for 3 days at 24 ^{o}C. At the end of the incubation period, the culture was centrifuged at 4000 g for 10 min to separate the fungal cells to get the cell free supernatant. The supernatant was assayed for flocculating activity.

2.3 Determination of Flocculating Activity

As described by Gao *et al.* (2006), 0.25 ml $CaCl_2$ (1.0 % w/w) was added to nine millilitre of kaolin clay (5 g/mL) in a McCartney bottle after which 0.1 ml cell free supernatant (CFS) was introduced and gently mixed for 1 minute. The mixture was vigorously stirred and allowed to stand for 5 min at room temperature. A reference tube in which the culture

supernatant (control) was replaced with distilled water was also included and measured under the same conditions. The final volume of the mixture was made up to 10 mL with distilled water. The optical density of the supernatant was determined using a UV spectrophotometer (Model Jenway6305) at 550 nm. Flocculation activity was determined using the following formula:

The flocculating activity was estimated from the formula below:

$$\text{Flocculating activity} = [(A - B)/A] \times 100\%$$

Where Aand B were optical densities of the control and samples respectively at 550 nm.

2.4. Optimization of culture conditions for bioflocculant production

The effect of different carbon and nitrogen sources on flocculating activity was assessed. Aliquots of 2 mL were inoculated into 200 mL of sterile BPB. The pH of the medium was adjusted to 7 and incubated at 30°C for 72 h with constant agitation speed of 160 rpm. After incubation the broth was centrifuged at 3,000 rpm for 30 min at 15°C and the cell free supernatant (CFS) was assessed for flocculation activity. Fructose, sucrose, lactose, maltose and starch were used as sole carbon sources, while the nitrogen sources evaluated were $(NH_4)_2SO_4$, NH_4COOH, and NH_3PO□ and the bioflocculants activity of the resulting CFS were assessed using the method of Lachhwani (2005).

2.5 Effects of Various Cations

Flocculant tests were conducted using the procedure stated above, but 1 % $CaCl_2$ solution was replaced by various metal ion solutions prior to measuring flocculating activity. Solutions of KCl, NaCl, (monovalent), $MgCl_2$ (divalent), $FeCl_3$ (trivalent) were used as salt sources.

2.6 Effects of Temperature

The effect of temperature on the bioflocculants was tested by measuring 2 mL of the seed culture broth into separate tubes and centrifuged at 4000 × g, 4°C for 30 min. The CFS of fungus grown in a Bioflocculant production broth (BPB) were transferred into clean 2 mL sterile Eppendorf tubes which were then incubated in a water bath at different temperatures ranging from 30°C to 100°C for 1 h. Flocculating activity was then determined at room temperature as previously stated (He *et al.,* 2004).

2.7 Time Course Experiment of the Bioflocculants

A modified method of Zhang *et al.* (2007) was used to determine the course of bioflocculant production. The test fungi were cultivated on PDA and the plates were incubated for 5 days at 26^{o}C. The spores produced were harvested by flooding the surface of the plates gently with 10 mL sterile water containing 2.0% Tween 80. The harvested fungal spores were standardized OD_{600} nm of 0.1. Five millilitre of the standardized spore suspension was inoculated into 150 mL of BPB in 500 mL flasks on a rotatory shaker (160 rpm) at 37°C. Ten millilitre of the culture was drawn at 24 h interval for a period of 7 days. The culture was centrifuged at 4000 g for 30 min, and the flocculating activity of the CFS was determined as stated above.

2.8 Effect of pH on Bioflocculant

To assess the effect of pH on the flocculating activity, it was determined by adjusting the pH of Kaolin clay suspension in a separate 250 mL flask and the pH values was adjusted using either NaOH or HCl (0.1 M) in the pH range of 3 to 10 and thereafter, the flocculating activity of each suspension at different pH value was determined as described by Kurane *et al.* (1994).

3.0 Results and Discussion

3.1 Isolation of Bioflocculant Producing Microorganisms

Two of the fourteen fungal isolates screened for bioflocculant activity showed and activities between 60% and 77%. These organisms were selected for further experimental purposes. Based on the morphological characteristics, the selected fungi were found to belong to the same genus: *Aspergillus niger* and *A. flavus*. Bioflocculant activity of *Aspergillus niger, A. flavus* has been recorded previously (Aljuboori *et al*., 2014, 2015). Table 1 shows the flocculating activity of the selected strains.

3.2 Effect of carbon source, nitrogen and cation on bioflocculant production

The effect of carbon sources on the test fungal strain's flocculating ability was tested. It was observed that glucose and sucrose were the most preferred carbon source for bioflocculant production with glucose inducing the highest flocculant production of 92% in *A. flavus* while maltose was the most preferred carbon source for bioflocculant production in *A. niger* inducing 77% production activity. Most microorganisms utilized for bioflocculant production in the literature preferred glucose as the sole carbon source (Cosa *et al*., 2013a; Nwodo *et al*., 2013) and this is also established in this report for *A. flavus.* Different fungi have been reported to make use of different carbon sources for the production of bioflocculants. Starch, sucrose and glucose were also reported as the favorable carbon sources for *Aspergillus parasiticus* in the production of bioflocculant by Deng *et al*. (2005) while Maltose was the carbon source of choice for *Solibacillus silvestris* (Wan *et al*., 2013). Aljuboori *et al*. (2013) observed that optimal production activity of 95% with for *A. flavus* which is in contrast to the observation in our study where sucrose only yielded 86 % as compared to glucose with 92 % (Fig. 1).

The effect of nitrogen sources on the test fungi revealed that the nitrogen source that favoured the optimum production of bioflocculant by both species of *Aspergillus* was peptone though *A. flavus* produced more (78%) and *A. niger* produced (62%). Most

microorganisms utilise either organic or inorganic nitrogen sources, or both, to produce bioflocculants. Piyo *et al.* (2011) reported that a *Bacillus* sp. Gilbert utilised an inorganic nitrogen source ammonium chloride effectively to produce bioflocculant with a flocculating activity of 91 %, while organic nitrogen sources like urea and peptone were poorly utilised as shown in Fig. 2. However, Li *et al*. (2013) and Aljuboori *et al.* (2013) reported that peptone was most suitable for bioflocculants production by *Paenibacillus elgii* and *Aspergillus flavus*.

It is well-established that cations are necessary to induce effective flocculation by increasing the initial adsorption of the bioflocculant on the kaolin clay suspension (Yim *et al*., 2007). In this study flocculation by *A. flavus* was stimulated by these cations, with $CaCl_2$ enhanced the strain to reach a flocculating activity of 78.4% while *A. niger* optimally produced in the presence of FeS showing 98 % activity (Fig. 3). However, Wu and Ye (2007) found out that Fe^{3+} inhibited bioflocculant production in the strains they investigated. The surface properties, specifically the distribution of charges on the surface of the bioflocculants lead to variety of ion valences being preferred by different bioflocculants-producing strains (Ntsaluba *et al*, 2013). Some authors however have reported that the bioflocculants produced by *A. flavus* showed outstanding flocculating activities for kaolin suspension in the absence of cations (Zhoa *et al*., 2013; Aljuboori *et al*., 2013)

3.3 Effect of Temperature on the Produced Bioflocculants

The effect of temperature on bioflocculant activity was observed that *A. flavus* bioflocculant had peaked at 40^{o}C (86%) but reduced afterwards as the temperature increased as shown in Fig. 4. A similar trend was also observed with *A. niger* but the highest activity was observed at 30^{o}C but a corresponding decrease in activity as the temperature increased although there was an increase at 60^{o}C. Aljuboori *et al.* (2013) found out that 40^{o}C was optimum temperature for bioflocculant produced by *A. flavus* strain. Pu *et al*. (2018) in their investigation using *A. niger* also observed a reduction in flocculating rate as temperature

increased from 30 to 80^{o}C but an increase afterwards, an observation similar to that obtained in this study. Bioflocculants with polysaccharide backbones tends to have a higher stability at increased temperatures as observed by Li *et al.* (2007) and Gong *et al*. (2008).

3.4 Effect of pH on Bioflocculant Produced

The pH of the environment is one of the most important external factors influencing the flocculating activity of a bioflocculant and the stability of the suspended particles (Wang *et al.,* 2011; Tang *et al*., 2014). A pH of 7 was shown to be optimum for the highest flocculating activity the bioflocculants produced by both *A. flavus* (97%) and *A. niger* (86 %) (Fig. 5). Pu *et al*. (2018) observed that the highest flocculating rate of 93.32 % was achieved at pH 6 for *A. niger* which is in contrast to the observation in this study.

3.5 Time Course of the Produced Bioflocculant

Most bioflocculants are usually produced during the exponential growth phase of microorganisms (Shih *et al*., 2001; Ugbenyen *et al*., 2012). The flocculating activity should increase gradually with an increase in cultivation time after which time it will reach a peak flocculation potential. Figure 6 shows the effect of incubation period on the bioflocculant produced by *A. flavus* and *A. niger.* It was observed that both *A. flavus* and *A. niger* produced bioflocculant maximally between 84 and around 80-96 hoursh. Okaiyeto *et al.* (2013, 2015) reported that *Bacillus* sp. had the highest flocculating potential of 83 % after 72 hours. The consequential decrease of the flocculating efficiency could be a product of cell autolysis and enzymatic activity.

3.6 The Physiochemical Properties of Treated and Untreated Water

It was observed that the conductivity and Total dissolved solids (TDS) of the water sample reduced when treated with the bioflocculants studied, with *A. niger* having higher value 255 than *A. flavus* with 196. In the same manner, the turbidity of the treatment water sample was lower than the control. The microbial count also showed a general reduction in the total bacterial count of the treatments as compared with the raw water samples with the

bioflocculants from *A. flavus* and *A. niger* reduced the bacterial load of the treated wastewater sample by 58.73% and 60.85% respectively. There was reduction in the conductivity of the water samples treated with both strains from 0.46 m/s in raw water to 0.25 m/s in *A. niger* bioflocculants. Therefore, the flocculant from *A. flavus* and *A. niger* seemed to have a fairly wide range of substrate specificity with strong activity and thus can be used in many industries

4.0 Conclusion

This study demonstrated the potential of two fungi species to produce effective bioflocculants which could widely be applied in different industrial processes including wastewater and downstream treatment.

ETHICAL: NA

CONSENT: NA

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Table 1: Flocculating activity of two bioflocculants produced

Properties	Organisms	
	A. niger	*A. flavus*
Optical density at 550nm	1.203±0.12	1.082±0.38
Flocculating activity	77.43±5.31	59.59±8.82
Flocculating activity (%)	77	60

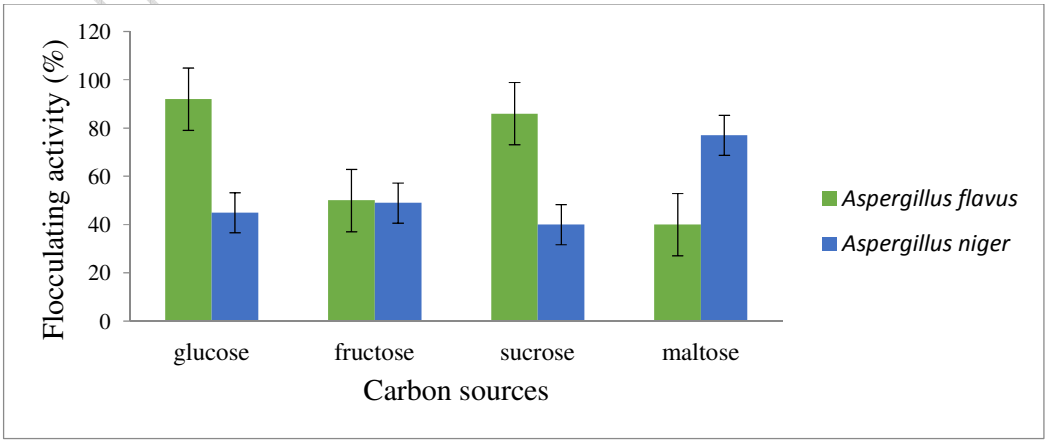

Figure 1: Effect of carbon source on bioflocculant produced by *Aspergillus flavus* and *Aspergillus niger*

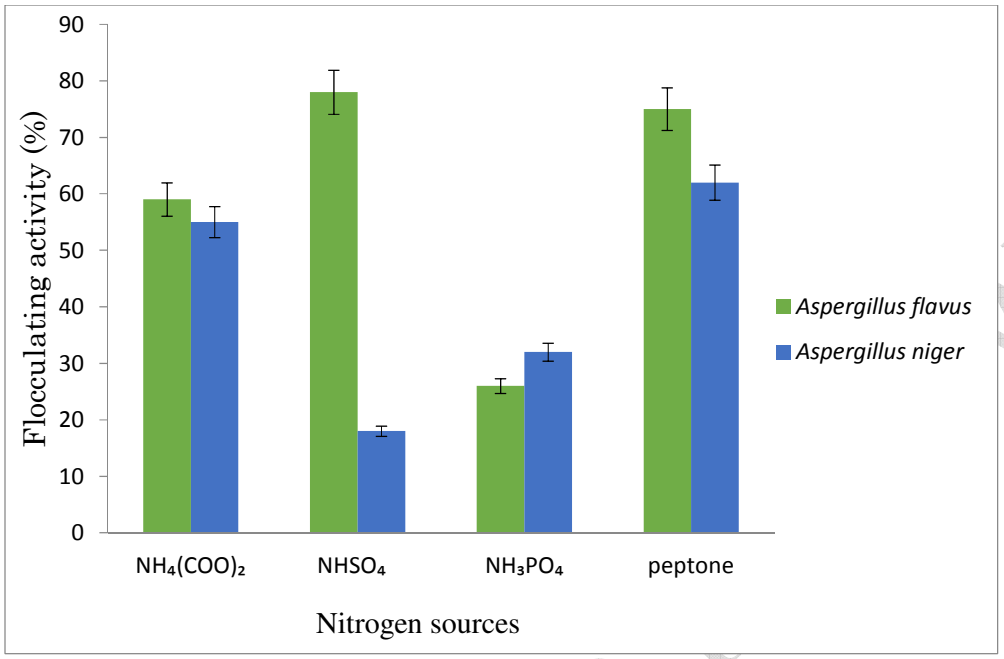

Figure 2: Effect of nitrogen source on bioflocculants produced by *A. flavus* and *A. niger*

correct $(NH_4)_2SO_4$

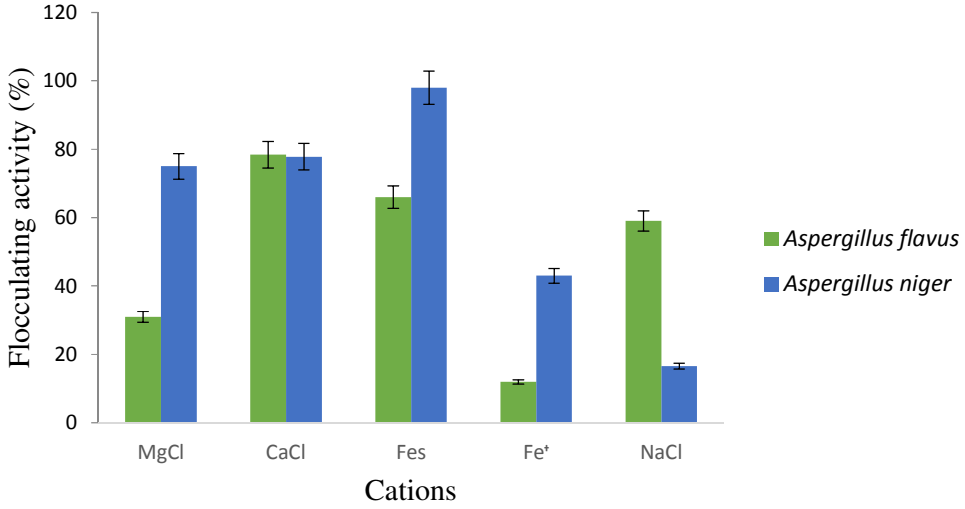

Figure 3: Effect of cation on bioflocculant produced by *Aspergillus flavus* and *Aspergillus niger*

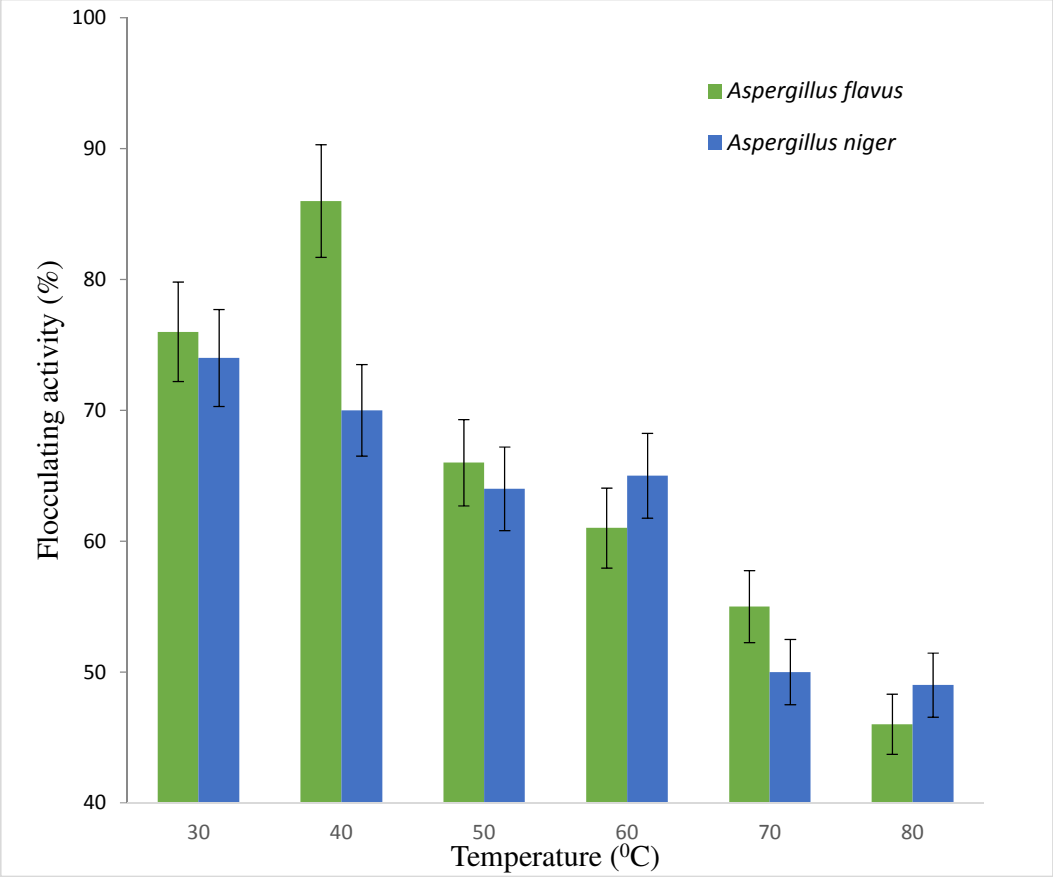

Figure 4: The effect of temperature on flocculating activity of *Aspergillus flavus* and *Aspergillus niger*.

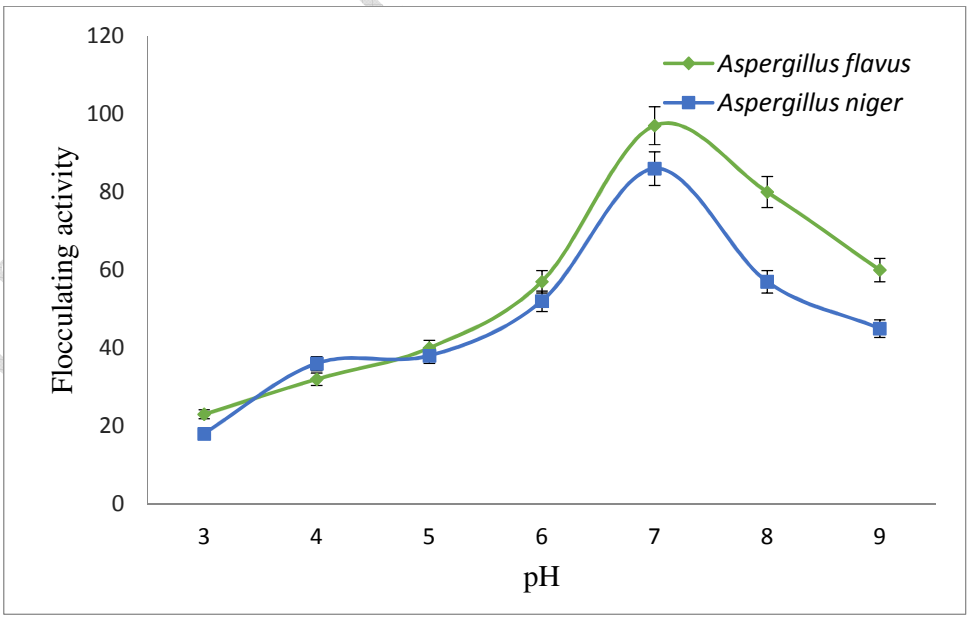

Figure 5: Effect of pH on flocculating activity of *A. flavus* and *A. niger*.

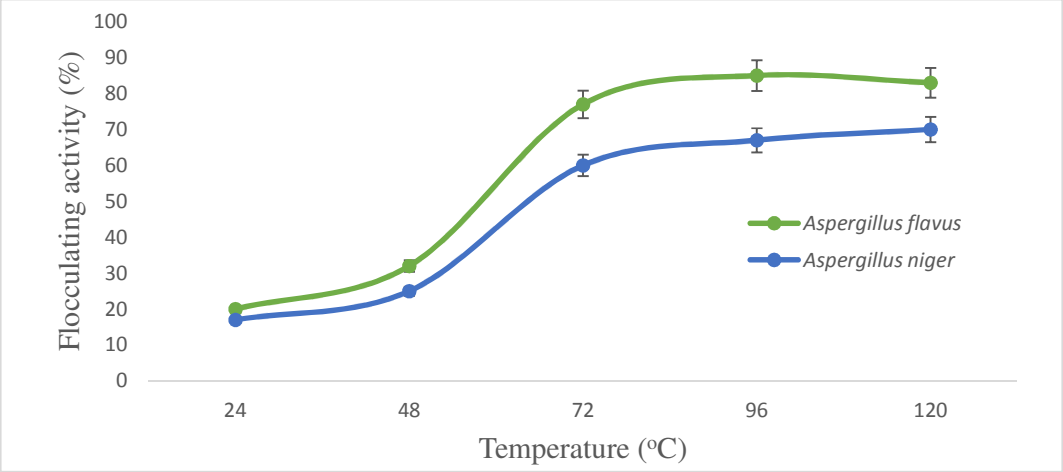

Figure 6: Effect of incubation period on bioflocculant produced by *A. flavus* and *A. niger*

Table 2: The effect of crude bioflocculants on the physiochemical parameter and bacterial load of the water samples

Parameters	Raw water	Bioflocculant sources (% decrease)	
		A. flavus	*A. niger*
Conductivity (m/s)	0.46±0.02	0.25±0.02 (45.65)	0.36±0.10 (21.74)
Total dissolved solids	267.00±8.90	196.00±16.00 (26.59)	255.00±12.10 (4.49)
pH	6.79±1.32	6.5±1.09 (4.27)	6.30±0.18 (7.22)
Turbidity	9.21±0.84	4.5±0.71 (51.14)	4.20±1.01 (54.40)
Log?TBC (log_{10} cfu/mL)	3.78±0.91	1.56±0.81 (58.73)	1.48±0.07 (60.85)

TBC = total bacterial count or log TBC