Flocculation behaviour of bioflocculant produced from chicken viscera

Jibrin Mohammed, Ndejiko[1,2], and Wan Rosmiza Zana Wan Dagang[2]

[1]Department of Microbiology, Ibrahim Badamasi Babangida University, Nigeria.
[2]Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia.

Abstract. The flocculation performance of bioflocculant produced by *Aspergillus flavus* S44-1 grown on chicken viscera hydrolysate was investigated. The investigations were carried out using jar testing and kaolin clay suspension as model wastewater. The bioflocculant yielded a minimum of 83.1% efficiency in flocculating 2–12 g L^{-1} kaolin clay suspension over a wide temperature range (4–80 °C) and functioned maximally at neutral pH. The bioflocculant significantly flocculated different suspended particles such as activated carbon (92%), soil solid (94.8%), and algae (69.4%) at varying concentrations. Bridging mediated by cation is suggested as the main mechanism of flocculation by the present bioflocculant.

1 Introduction

The discharge of untreated industrial, agricultural, and domestic wastes constitutes a major source of water pollution. Flocculation of suspended particles from wastewater is one of the major ways of addressing water pollution. Over time, synthetic chemical flocculants have extensively been used for removal of suspended particles in water due to their efficiency and cost effectiveness; however, these flocculants are associated with secondary pollutant accumulation that may be toxic to human health [1].

In search of an alternative to chemical flocculants, extensive research effort has been devoted to biological synthesis of flocculants, which is known as bioflocculant. Recently, bioflocculants are produced through microbial fermentation by various microorganisms such as bacteria, actinomycetes, fungi, and algae [2]. Inadequate knowledge on flocculating conditions of any newly synthesised bioflocculant could result in poor efficiency. Thus, the economics of bioflocculant application is coupled with the use of low-cost substrate for microbial fermentation and identification of flocculating conditions of any newly synthesised bioflocculant [3]. The use of industrial/agricultural waste as a nutrient source for microbial fermentation has a concurrent advantage of combating environmental pollution by such wastes and thus reducing the cost of bioflocculant production and application. The present study is an evaluation of flocculation performance of a bioflocculant produced by *Aspergillus flavus* S44-1 grown on chicken viscera hydrolysate as a sole source of nutrient.

2 Materials and methods

2.1 Microorganism and culture conditions for bioflocculant production

Aspergillus flavus S44-1 [4] was obtained from Microbial Culture Collection Unit of Universiti Putra Malaysia. The organism was reactivated from stock culture and cultured on potato dextrose agar until there was spore formation. The spores were harvested with 5% glycerol and spore concentration was adjusted to 1.0×10^6 spore ml^{-1} with the aid of a haemocytometer. The spores were subsequently inoculated into sterile liquid of chicken

viscera hydrolysate (containing crude protein (5.40), sugar (3.20), carbon (5.86), nitrogen (1.27), sulphur (0.83), and hydrogen (10) (all in %w/w)) as the production medium. The fermentation was carried out under previously optimised culture conditions (incubation time of 72 h, pH 7, shaker speed of 150 rpm, temperature of 35 °C, and inoculum of 4%) for *A. flavus* growth and bioflocculant production.

2.2 Preparation of bioflocculant

The bioflocculant was prepared and purified in accordance with the methods demonstrated by Aljuboori, Idris [4] and Sun, Lin [5].

2.3 Measurement of flocculation efficiency

The flocculation efficiency of the bioflocculant was determined using 4 g L^{-1} kaolin clay suspension following the techniques demonstrated by More, Yan [6], Czemierska, Szcześ [7] and Xia, Liang [3]. Different volumes of crude bioflocculant were added to 100 ml of kaolin suspension together with 3 ml of 1% calcium chloride as the bioflocculant aid in a 500 ml glass, and the pH was adjusted to a neutral point. The mixture was stirred in a flocculator tester at 200 rpm for 1 min, slowly stirred at 80 rpm for 5 min, and then held for 5 min using a 6-breaker jar tester (JLT6, VELP Scientifica, Italy). The optical density (OD) of the clarified upper solution was measured with a spectrophotometer (T60 spectrophotometer) at 550 nm. The flocculation rate was finally estimated in accordance with the following equation [8]:

$$\text{Flocculation Efficiency} = [(A - B/A) \times 100\%] \quad (1)$$

Where A represents the absorbance of the control (where sterile viscera medium was used to replace the bioflocculant) at 550 nm and B is the absorbance of the sample at 550 nm.

2.4 Factors affecting flocculation efficiency

The effects of pH values of kaolin clay suspension (3–12), temperature (4–80 °C), and kaolin clay concentration (2–12 g L^{-1}) were investigated following the same procedure as stated above. All experimental trials were conducted in triplicate.

3 Results and discussion

3.1 Bioflocculation relationship among kaolin clay, bioflocculant, and Ca^{2+}

Table 1 presents bioflocculation relationship among kaolin clay, bioflocculant, and Ca^{2+}. The flocculation efficiency of 41.47% was achieved when only Ca^{2+} was present as the flocculant for kaolin clay suspension. This is due to the partial lack of stability of the particles brought about by the ability of Ca^{2+} to compress the charged double layer of clay particles and thus decreases the electrostatic repulsion among the particles. The flocculation efficiency of 60.33% was recorded when only the bioflocculant was present in kaolin suspension. The presence of amino and carboxylate groups in the bioflocculant facilitates adsorption of the bioflocculant on kaolin clay surfaces, thereby allowing floc formation [8]. The efficiency rose to 91.26% when the bioflocculant was used simultaneously with Ca^{2+}. The zeta potential also decreased rapidly from -32.10 to -2.9 mV. This shows the bioflocculant is stimulated by Ca^{2+}. Earlier characterisation of this bioflocculant revealed the presence of negatively charged groups such as uronic acids, amine, and carboxylic groups. Neutralisation of these groups found in the bioflocculant by Ca^{2+} confers much positivity on the bioflocculant to facilitate the flocculation of negatively charged kaolin particles [8].

Table 1. Bioflocculation relationship among kaolin clay, bioflocculant, and Ca^{2+}.

	Kaolin Clay Only	**Kaolin Clay + Ca^{2+}**	**Kaolin + Biofloc.**	**Kaolin + Ca^{2+} + Biofloc.**
Absorbance	0.949	0.546	0.389	0.078
Flocculation Efficiency (%)	0.66	41.47	60.33	91.26
Zeta Potential (mV)	-32.10	-12.88	-16.95	-2.9

3.2 Effect of kaolin clay concentration on flocculation efficiency

The effect of kaolin clay concentration on bioflocculant efficiency is summarised in Figure 1. The bioflocculant achieved over 90% efficiency over a wide range of kaolin clay concentrations (2–6 g L^{-1}) but as the kaolin clay concentration increased to 8 g L^{-1} and above, the efficiency decreased slightly to below 90%. The decrease in efficiency can be attributed to higher kaolin clay concentration, which limits the ability of bioflocculant to neutralise and destabilise residual particles [9]. The ability of lower concentration of bioflocculant to flocculate colloidal particles from a wide range of concentrated wastewater is cost effective and desirable for its industrial application. The ability of this bioflocculant to retain flocculation efficiency to about 83% even at 12 g L^{-1} is an indication of a good flocculating agent that can be applied in removing colloidal particles from various forms of wastewater.

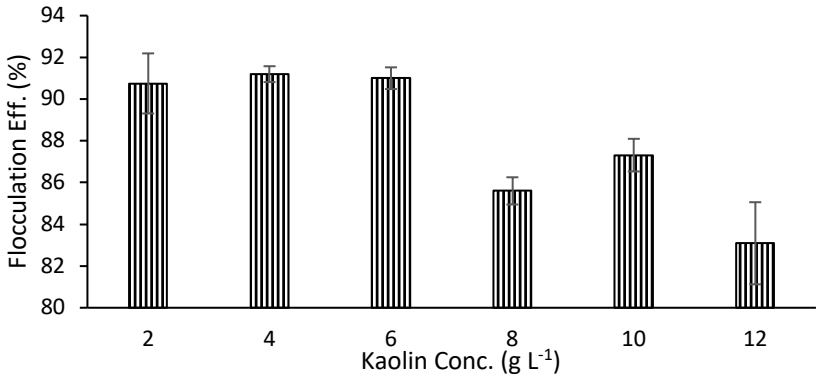

Fig. 1. Effect of kaolin clay concentration on bioflocculant.

3.3 Effect of temperature on flocculation efficiency

The thermostability of a bioflocculant is a favourable property for its industrial exploitation. The effect of temperature on flocculation efficiency of the bioflocculant is shown in Figure 2. It retained the maximum efficiency of 90.3% within the temperature range of 4 to 40 °C and decreased to a minimum of 84.8% as the temperature rose to between 60 and 80 °C. The maximum efficiency was achieved at 20 °C. The thermostability of this bioflocculant may be attributed to its polysaccharide backbone [10]. The characterisation of this bioflocculant in our previous study revealed the presence of 23.46% protein and 74.5% sugar, including 46% neutral sugar that can serve as a polysaccharide backbone and 2.01% uronic acid. Less thermostable bioflocculants are mainly composed of proteins that can easily degrade at high temperatures [11]. This finding correlates with the reports of He, Zou [12]

and Li, Chen [13], who reported the thermostability of the bioflocculants produced by *Halomonas sp. V3a* and *Bacillus licheniformis X14*, respectively.

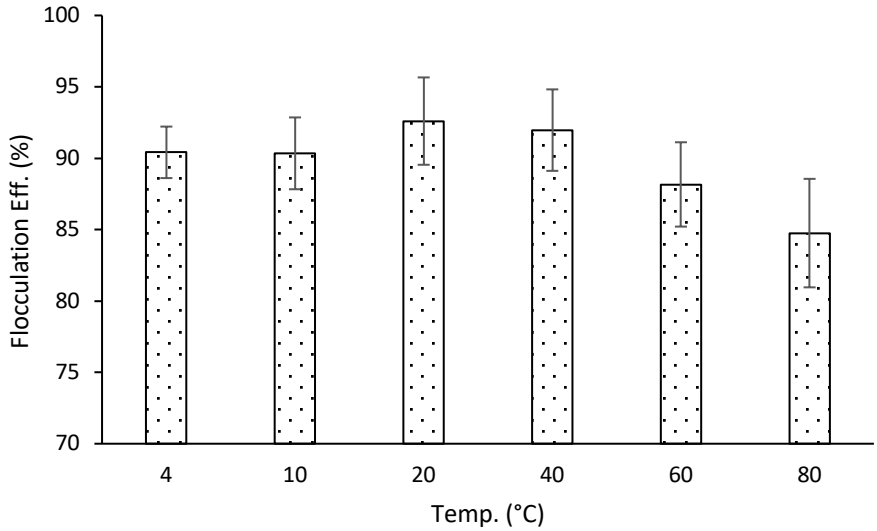

Fig. 2. Effect of temperature on the flocculation efficiency of bioflocculant.

3.4 Effect of pH on flocculation efficiency

Generally, pH of wastewater has been identified as one of the most important factors that can easily influence the flocculation activity of any given bioflocculant [14]. The result (Figure 3) shows that pH 5 to 9 are favourable for flocculation with the optimum efficiency of 91.5% at pH 7. The drop in efficiency is due to the ability of OH^- ions to impede complex formation between the bioflocculant and kaolin particles. The flocculation efficiency of the bioflocculant produced by *Paenibacillus elgii* was above 80% in the pH range of 3 to 11 [15]. A shift in the pH of wastewater can modify the ionic charge status of the bioflocculant and change the surface properties of suspended colloids [1]. The disparity in the pH requisite of reaction mixture may be associated with the characteristics of bioflocculant that show varying electric states at different pH [16].

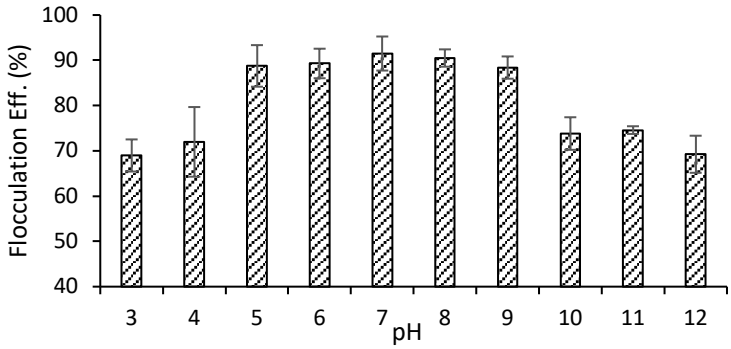

Fig. 3. Effect of pH of the suspension on the flocculation efficiency of bioflocculant.

3.5 Flocculation of different types of suspended solids

Figure 4 presents bioflocculation efficiency on kaolin clay solution, suspended soil solid, algae solution, and activated carbon by the bioflocculant using jar test. The results indicated that 5–6 ml of crude bioflocculant produced about 94% flocculation efficiency in 4 g L^{-1} soil solution. For kaolin clay suspension (4 g L^{-1}) and activated carbon suspension (4 g L^{-1}), 4 and 9 ml of crude bioflocculant produced high flocculation efficiency of 92.8% and 92.0%, respectively. The highest flocculation efficiency of approximately 70% was recorded for algae suspension with 4–10 ml of crude bioflocculant. The flocculation efficiencies achieved for soil solution, activated carbon suspension, and kaolin clay suspension are due to high flocculation property of the bioflocculant and adsorption of the suspended solids. Activated carbon, for instance, has broad adsorption and affinity for polysaccharides and metals to form complexes [9, 17].

The present bioflocculant is better suited for the flocculation of activated carbon at neutral pH than bioflocculant MBFF19 that produced efficiency of 90% at acidic pH [18]. In this study, the flocculation efficiency on activated carbon increased steadily as bioflocculant dose increased from 1 to 9 ml and dropped at 10 ml. For soil solid suspension, the efficiency reached the maximum when the crude bioflocculant dose was between 4 and 7 ml. About 120 mg L^{-1} of *Aspergillus sojae* bioflocculant was needed to flocculate up to 94% of yeast cells at neutral pH [19]. Overall, these findings indicate that the bioflocculant can serve as an alternative to chemical flocculants in soil solid and activated carbon removal at neutral pH.

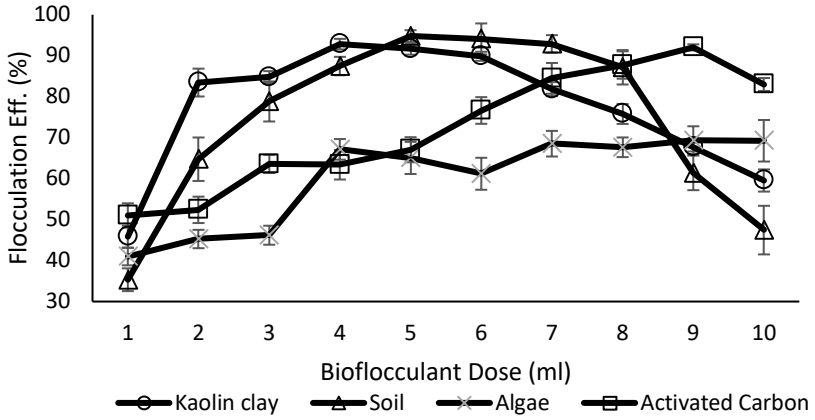

Fig. 4. Flocculation of different suspended particles by the bioflocculant.

4 Conclusion

The bioflocculant produced by *A. flavus* grown on chicken viscera hydrolysate was found to retain its flocculation activity over a wide range of temperature and pH (i.e., 5–9). Moreover, the bioflocculant could flocculate different concentrations of kaolin clay suspension, soil solid, and activated carbon. Therefore, this bioflocculant has the potential to be used in water treatment industries as a replacement for chemical flocculants.

The authors acknowledge Universiti Teknologi Malaysia and GUP Tier 1 (Q. J130000.2545.13H22) and Demand-Driven Innovation grant (R. J130000.7845.4L190) for their financial support, and also Pakar Go Green Sdn. Bhd for supplying the fungus strain used in this study.

References

1. K. Okaiyeto, et al., Implications for public health demands alternatives to inorganic and synthetic flocculants: bioflocculants as important candidates. Microbiol.Open. (2016).
2. H. Zimmermann, S. G. Shirley, and U. Zimmermann, *Alginate-based encapsulation of cells: past, present, and future.* Curr Diabetes Rep **7**(4): 314. (2007).
2. X. Xia, et al., Production and flocculating properties of a compound biopolymer flocculant from corn ethanol wastewater. Bioresour. Technol.. **247**: 924-929. (2018).
4. A. H. R. Aljuboori, et al., Production and characterization of a bioflocculant produced by *Aspergillus flavus*. Bioresour. Technol.. **127**: 489-493. (2013).
5. P.-F. Sun, et al., Preparation of a new-style composite containing a key bioflocculant produced by *Pseudomonas aeruginosa* ZJU1 and its flocculating effect on harmful algal blooms. J. Hazard. Mater.. **284**: 215-221. (2015).
6. T. T. More, et al., Biopolymers Production by Mixed Culture and Their Applications in Water and Wastewater Treatment. Water Environ Res. **87**(6): 533-546. (2015).
7. M. Czemierska, et al., Characterisation of exopolymer R-202 isolated from *Rhodococcus rhodochrous* and its flocculating properties. Eur Polym J. **88**: 21-33. (2017).
8. Z. Wang, et al., Flocculation Characterization of a Bioflocculant from *Bacillus licheniformis*. Ind. Eng. Chem. Res. **54**(11): 2894-2901. (2015).
9. A. H. R. Aljuboori, et al., Flocculation behavior and mechanism of bioflocculant produced by *Aspergillus flavus.* J Environ Manage **150**: 466-471. (2015).
10. K. Okaiyeto, et al., Implications for public health demands alternatives to inorganic and synthetic flocculants: bioflocculants as important candidates. MicrobiologyOpen. **5**(2): 177-211. (2016).
11. W. Liu, et al., Production and characterization of an intracellular bioflocculant by *Chryseobacterium daeguense* W6 cultured in low nutrition medium. Bioresour. Technol.. **101**(3): 1044-1048. (2010).
12. J. He, et al., Characteristics and flocculating mechanism of a novel bioflocculant HBF-3 produced by deep-sea bacterium mutant *Halomonas* sp. V3a'. World J Microbiol Biotechnol. **26**(6): 1135-1141. (2010).
13. Z. Li, et al., Characterization and flocculating properties of a novel bioflocculant produced by *Bacillus circulans*. World J Microbiol Biotechnol **25**(5): 745. (2009).
14. H. Salehizadeh and N. Yan, Recent advances in extracellular biopolymer flocculants. Biotechnol. adv. **32**(8): 1506-1522. (2014).
15. O. Li, et al., Optimization and characterization of polysaccharide-based bioflocculant produced by *Paenibacillus elgii* B69 and its application in wastewater treatment. Bioresour. Technol.. **134**: 87-93. (2013).
16. A. Ugbenyen, et al., Bioflocculant production by *Bacillus* sp. Gilbert isolated from a marine environment in South Africa. Appl biochem microbiol. **50**(1): 49-54. (2014).
17. J. Charles, et al., Pollutant removal from industrial discharge water using individual and combined effects of adsorption and ion-exchange processes: Chemical abatement. J Saudi Chem Soc. **20**(2): 185-194. (2016).
18. Y. Zheng, et al., Production and characteristics of a bioflocculant produced by *Bacillus* sp. F19. Bioresour. Technol.. **99**(16): 7686-7691. (2008).
19. Nakamura, J., Miyashiro, S., and Hirose, Y. Modes of flocculation of yeast cells with flocculant produced by Aspergillus sojae AJ7002. *Agric. Biol. Chem., 40*(8), 1565-1571. (1976).