Effect of Optimization of Cultural Parameters on Exobiopolymer Production by Microbial Isolates and Their Application in Wastewater Treatment

Gboyega Ebenezer Adebami[1,2*], Bukola Christiana Adebayo-Tayo[1] and Akinyemi Akinniyi Akinyugha[3]

[1]*Department of Microbiology, University of Ibadan, Oyo State, Nigeria.*
[2]*Department of Biological Sciences, Mountain Top University, Ogun State, Nigeria.*
[3]*Department of Chemical Sciences, Mountain Top University, Ogun State, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Authors GEA and BCA designed the study, performed the statistical analysis and wrote the protocol, Author GEA wrote the first draft of the manuscript and managed the literature searches. Author AAA provided the technical support and proof read the manuscript. All the authors managed the analyses of the study, read and approved the final manuscript.

Article Information

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ABSTRACT

The optimization of culture media and conditions for efficient production of exopolymers by microorganisms cannot be overemphasized. As such, this work investigated the effect of optimizing culture media on bioflocculant production using *Alcaligenes aquatilis* AP4 and *Bacillus clausii* NB2 isolated from wastewater samples. It also studied the application of the resulting bioflocculants for treatment of wastewater. The basal medium used for the batch fermentation was supplemented with different concentrations of carbon and nitrogen sources. The bioflocculants produced were tested on kaolin solution, brewery and palm-oil effluents in concentrations ranging from 100-1000 mg/L. It was

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*Corresponding author: E-mail: gboyega.adebami@gmail.com;

observed that optimization of culture media by the two strains had a significant effect ($P \le 0.05$) on bioflocculant production. At 72 hours of incubation, 15.0 and 20.0g/l glucose concentration gave the best conditions for bioflocculant production by isolates AP4 and NB2 respectively. In addition, 0.50 and 0.30 g/l of inorganic nitrogen source ($(NH_4)_2SO_4$) and 0.70g/L of organic nitrogen (Urea) were required by both isolates. 10 and 15ml/L of bioflocculant dosage respectively gave the best flocculating activities. The ability of the bioflocculants to flocculate kaolin solution, brewery wastewater and palm-oil effluent were confirmed. In addition, the bioflocullants also showed a tremendous ability to reduce COD and turbidity of wastewater.

1. INTRODUCTION

Biopolymers could be grouped into either intracellular or extracellular [1]. Extracellular polymers (exobiopolymers) are high-molecular weight mixtures of polymers consisting of long-chains of repeating unit of sugar residues which are produced and released into the external environment. Some naturally occurring biopolymers are chitosan, cellulose, guar gum, sodium alginate, starch, tannin, and microbial polymers which sometimes secrete their biopolymer into their surrounding environment [2]. Some of these microbes synthesize their biopolymer in form of bioflocculant which could be polysaccharides [3-6], glycoprotein [7-9] or functional proteins [10-11].

These bioflocculant-producing organisms are generally present in environmental samples such as soil [12-13], activated sludge [14-15], crude oil [16], wastewaters [17-18], and lignocellulosic biomass [19]. Generally, microbial flocculants can be produced by eubacteria, actinomycetes and fungi during their growth. They are preferred to the conventional inorganic and organic flocculants because of their biodegradability and their high flocculating capability. In addition, they are generally harmless to humans and the environment, safe for ecosystems and they do not generate any significant secondary pollution [20-21]. This bioflocculant can be used in industrial and wastewater treatment processes such as pharmaceutical, fermentation, food industries dredging and downstream processing [22-23].

Specifically, bioflocculants have been applied in various ways and found to be potent in the removal of heavy metals and dyes. They also reduce the turbidity and COD of effluents [14, 24] in addition to increasing precipitation of inorganic solid particles from suspensions [25-26], treatment of drinking water [27], treatment of oil-polluted wastewaters and landfill leachate [28].

However, the high cost of bioflocculant has been one of the limiting factors towards its application [29]. As such, different culture media have been experimented as substrates for bioflocculant production to cushion this effect including dairy and sauce wastewaters, effluent from a hydrogen-producing bioreactor [30-32]; sewage, brewery and palm-oil effluents [33]; lignocellulosic biomass [34]; soybean juice and fishmeal wastewaters [30,35]. Alternatively, Effective optimization will also reduce the problem created by high cost of production. Optimization of culture conditions such as carbon and nitrogen sources, bioflocculant dosage, pH and temperature could lead to a more efficient flocculation. This research work is carried out to determine the effect of optimizing certain culture parameters on bioflocculants production and the application of the produced bioflocculants to the treatment of wastewater samples.

2. MATERIALS AND METHODS

2.1 Culture Preparation

Isolation and characterization of bioflocculants producing isolates *Alcaligenes aquatilis* AP4 and *Bacillus clausii* NB2 obtained from palm oil mill effluent and brewery wastewater respectively in south-western part of Nigeria had been previously reported [36]. The strains were preserved in 20% glycerol stock at −80°C. 16SrDNA sequence determination and phylogenetic analysis of the two strains showed that AP4 and NB2 were *A. aquatilis* and *B. clausii* respectively.

The stock cultures were re-isolated and maintained on nutrient agar, incubated at 30°C for 72 h and stored at 4°C. The seed culture was grown in a 250 ml flask containing: nutrient broth, 10.0 g; potato dextrose broth, 5.0 g; glycerol, 3 ml; yeast extract, 6.5 g; sodium chloride, 1.0 g in 1 litre of distilled water. The pH of the medium

was adjusted to 7.0 and the medium was autoclaved and inoculated with pure culture from the stock culture and incubated for 24 hrs.

2.2 Bioflocculant Production

The isolates were used for bioflocculant production using bioflocculant production broth medium (BPB). The BPB composition include: 10 g glucose, 2 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 NaCl, 0.5 g $CaCO_3$, and 0.5 g yeast extract. The mixture was dissolved in 1 liter deionized water with the initial pH adjusted to 7.0. The medium was sterilized, inoculated with pure culture of the isolates and incubated on a rotary shaker at 120 rpm and 37°C for 3 days. Kaolin suspensions at a concentration of 5,000 mg/l were used to evaluate the flocculating abilities of the produced exopolysaccharides [11].

2.3 Single and Consortium Culture

The two isolates were used separately and as consortium. The inoculum of the two isolates were first grown aerobically in 250ml Erlenmeyer flasks at 25°C on a rotary shaker at 120rpm for 72 hrs. 1 ml of each strain were then inoculated into a fresh 250 ml Erlenmeyer flask containing culture broth. The medium was incubated at 25°C on a rotary shaker at 120 rpm for 72 hrs, after which the fermentation broth was centrifuged at 4000 × g for 30 minutes at 4°C and the supernatant was used for determination of flocculating activity using Kaolin suspension at concentration of 5,000 mg/l. The optical density (OD) of the clarifying upper phase solution was measured at 550 nm with a UV spectrophotometer [37].

2.4 Determination of Flocculating Activity

The flocculating activity was determined according to the method of Kurane et al. [38] as modified by Gao et al. [20]. A suspension of kaolin clay was used as test material for flocculating activity determination. The kaolin clay was suspended in distilled water at a concentration of 5 g/L at pH 7.0 and used as a stock solution for the subsequent assays. The following solutions were mixed in a test tube: kaolin clay suspension (9 mL), culture supernatant (0.1 mL) and 1% $CaCl_2$ (0.25 mL). A reference tube in which the culture supernatant was replaced with distilled water was also included and measured under the same conditions. The final volume of all mixtures was made up to 10 mL with distilled water. After

mixing gently, the solutions were allowed to settle for 5 min. at room temperature. The optical density (OD) of the clarifying upper phase solution was measured at 550nm with a UV spectrophotometer and the flocculating activity determined as follows:

Flocculating rate (%) = [(B − A) / B] × 100%

Where A and B are optical densities at 550 nm of the sample and control respectively.

2.5 Optimization of Cultural Parameters for Bioflocculant Production

Bioflocculant production by the isolates was carried out under optimized conditions. The basal medium was supplemented with different concentrations of the best carbon source (5.0-30.0 g/L), nitrogen sources (0.05-1.0 g/L) and dosage (5.0-30.0 ml/L). The medium was inoculated and incubated under shaking (120 rpm) condition. After incubation, the kaolin assay was carried out to check the maximum flocculating activity of the isolates, after which the absorbance was read using a spectrophotometer at a wavelength of 550 nm.

2.6 Application of Bioflocculants in the Treatment of Wastewaters

Wastewaters used for this analysis were collected from two different states in south-western part of Nigeria. The brewing effluent was collected from International Breweries Plc Omiasoro, Ilesha, Osun State while palm oil effluent was collected from Fiditi village, Oyo State. The Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), turbidity, pH, conductivity, salinity and temperature of the wastewater samples were determined. All wastewaters were stored at 4°C before flocculation. Varied concentrations (100-1000 mg/L) i.e. (0.1-1.0 ml/l) of bioflocculant and 1 ml of 1% $CaCl_2$ solution was added to the 100 ml wastewaters at pH 7.0. The compound was mixed thoroughly and allowed to settle for 5 minutes, after which the optical density (OD) of the clarifying upper phase solution was measured at 550 nm with a UV spectrophotometer and the flocculating activity determined. The dissolved oxygen, chemical oxygen demand (COD) and turbidity of the wastewaters were measured using Dissolved Oxygen meter (Extech Instrument), multi-parameter photometer (Hanna) and microprocessor turbidity meter (Hanna) respectively. The removal efficiency of COD and

turbidity of the brewery and palm oil wastewaters were determined using the method of Gong et al. [4]. The results obtained were compared with chemically synthesized flocculants in which the bioflocculant was replaced with Aluminium sulphate (Alum).

2.7 Statistical Analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) to determine their significance at $P \leq 0.05$. Tukey-Kramer test method was used. All data were treated in replicates, the standard deviation of the mean values was taken [39].

3. RESULTS AND DISCUSSION

BLAST (Basic Local Alignment Search Tool) analyses of the 16SrRNA gene nucleotide sequence of the bacterial strain, coded AP4 and NB2, showed 95 and 97% similarities to *A. aquatilis* (accession number KT748636) and *B. clausii* (accession number HM560954) respectively. A phylogenetic tree was constructed between them and similar sequences found in GenBank as shown in Fig. 1.

3.1 Effect of Single Starter/Consortium on Bioflocculant Production by the Isolates

The production of bioflocculant and its activity by single starter and consortium isolates are shown in Fig. 2. It was observed that there was a significant difference ($P \leq 0.05$) in the flocculating activity using the strains singly and in consortium. The flocculating activity of the single and combined starter ranged from 54.31^c - 82.66^a%, 58.10^c - 79.84^a% and 50.15^c - 80.66^a% for *A. aquatilis* AP4, *B. clausii* NB2 and Consortium respectively. At the end of 72 hrs of incubation, the highest flocculating activity was 82.66^a% in *A. aquatilis* AP4, 79.84^a% in *B. clausii* NB2 and 80.66^a% in their consortium. This finding was similar to the report of Gül and Dönmez, [40] on the decolourization of Remazol Blue dye by *Aspergillus versicolor* and *Rhizopus arrhizus* both as individual and mixed culture. At the end of incubation period, 46.3, 89.4 and 86.5% activities were observed for *R. arrhizus*, *A. versicolor* and their mixed culture respectively. Maliehe et al. [37] observed an increased yield when the consortium of *B. pumilus* JX860616 and *A. faecalis* HCB2 were used for bioflocculant production. Ntsaluba et al. [41] reported 89% flocculating activity for the consortium of *Methylobacterium* sp. Obi and *Actinobacterium*

sp. Mayor. However, Menetrez, [42] and Wang et al. [43] reported that a lower activity of consortium may sometimes be noticed because synthetic microbial consortia are often fragile and unstable, which limit their applications in industrial biotechnology in contrast to what is obtained in their environment. Kim et al. [44] observed that mixed cultures created by arbitrarily combining different species, population compositions are often unstable and prone to domination by a single species or extinction.

Recently, there has been growing interest in the biotechnological consortia synthesis of isolates and their industrial applications. Natural microbial consortia hold many appealing properties in a bioprocessing context, such as stability, functional robustness, and the ability to perform complex tasks [45]. For instance, microbial consortium have been earmarked as a reliable and efficient alternative to single strains for lignocellulose degradation [46]. Cortes-Tolalpa et al. [47] used an interesting approach by varying environmental sources of microbial inocula in the lignocellulose-degrading of wheat plant by using up to six organisms as consortia. These approach gave rise to the fact that microbes sometimes live in synergistic communities in most natural environments in which individual species with specialized roles cooperate to survive and thrive together [48]. Gül and Dönmez [40] reported that addition of surfactant such as dodecyl trimethyl ammonium bromide (DTAB) in small concentration increases the activity of consortium organisms.

3.2 Effect of Glucose Optimization on the Flocculating Activities of the Isolates

Fig. 3 shows the effect of varied concentrations of glucose on bioflocculant production by AP4 and NB2. At 72 hrs of incubation, there was a significant difference ($P \leq 0.05$) in the flocculating activity of different glucose concentration, which ranged from 77.44^f - 89.66^a% and 58.79^f - 80.23^a% for isolates AP4 and NB2 respectively. It was discovered that isolate AP4 had its highest flocculating activity at 15.0 g/l of glucose concentration while NB2 had its highest at 20.0g/l. This finding was similar to the report of Okaiyeto et al. [49] that 20g/L of glucose was required by *Bacillus.* sp. AEMREG7 to obtain the best flocculating activity of 88.03% among several carbon sources tested. Luo et al. [50] reported that 25 g/L of glucose concentration was required by bioflocculant-producing *Bacillus megaterium* SP1 to obtained 90.6% flocculating

activity. Liu et al. [51] report on alkali-tolerant strain of *Microbacterium esteraromaticum* C26 showed that glucose gave the best among the carbon sources tested at concentration 3g/L which is in contrast to the present study.

Minty et al. [52] and Wang et al. [43] explained that the backbone of functional biological molecules is carbon, which could occur as monosaccharides, disaccharides, polysaccharides, or alcohol sugar. Glucose is a monosaccharide, which provides faster growth than other sugars, and is mostly consumed first in sugar mixtures [53]. Moreover, when added to nutrient broth, it may increase the overall growth rates and biomass of bacteria over time [54]. In addition, there have been various reports of complex sugars being used for bioflocculant production. For instance, Li et al. [55] reported that starch was found the most favourable by *Oceanobacillus polygoni* in the production of bioflocculant MBF-HG6. Alternatively, glucose may be inhibitory to cell growth if the concentration is too high [56-58]. Increasing the amount of glucose have been shown to limit microbial growth by inhibiting proteinaceous enzymes [59-60].
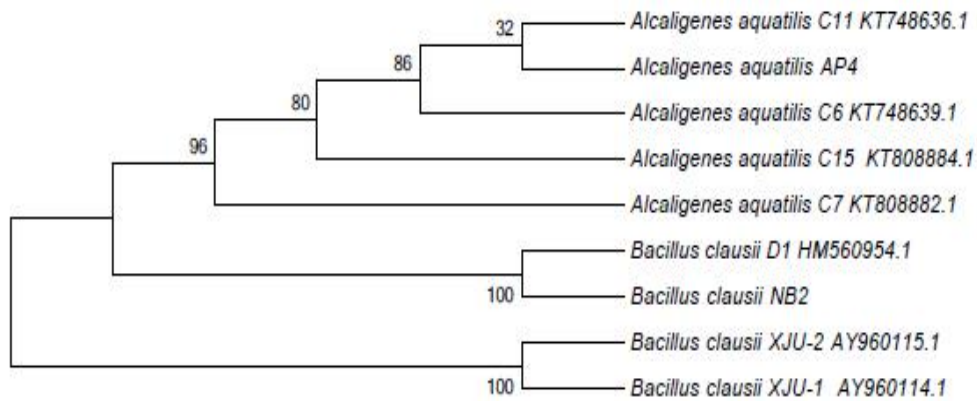

Fig. 1. Phylogenetic tree showing the relationships between *A. aquatilis* AP4 as well as *B. clausii* NB2 strains and other closely related species collected from the Gene Bank. The dendogram was generated by the neighbor-joining method. Bootstrap values per 100 bootstrap analysis presented for values greater than 50%
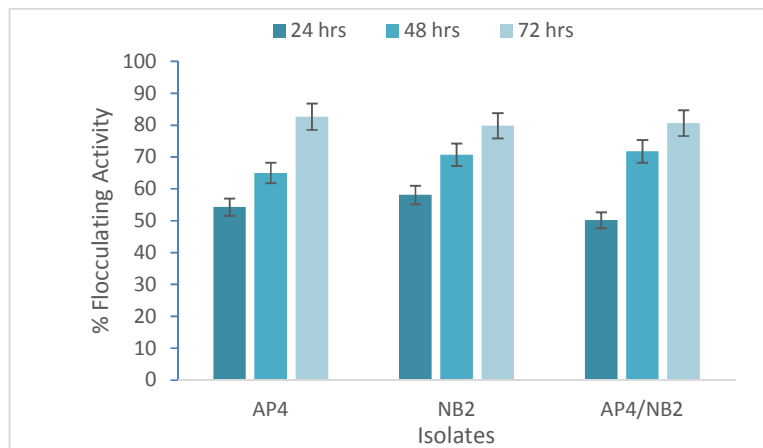

Fig. 2. Effect of single and combined starter on the bioflocculation productions
Key: AP4= A. aquatilis AP4; NB2= B. clausiiNB2 and AP4/NB2= Consortium by A. aquatilis AP4 and B. clausii NB2
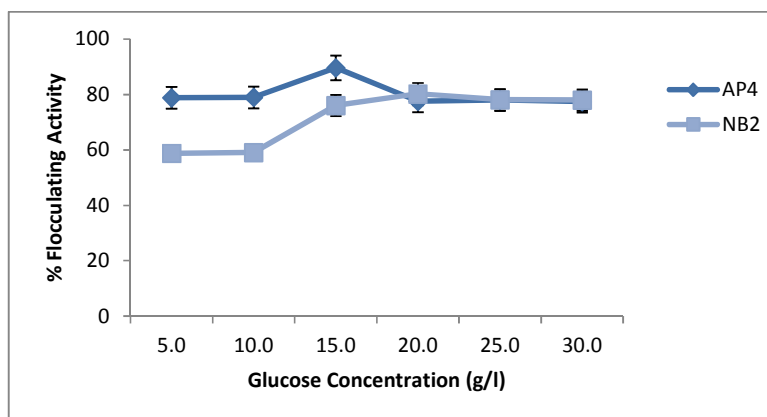
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Fig. 3. Effect of different conc. of glucose on the flocculating activities of AP4 and NB2

3.3 Effect of Optimization of Nitrogen Sources on the Flocculating Activities of the Isolates

The effect of different concentrations of organic (urea) and inorganic (ammonium sulphate) nitrogen sources on the flocculating ability of the isolates were investigated. Fig. 4 shows the effect of varied concentrations of ammonium sulphate on bioflocculant production by AP4 and NB2. There was a significant difference (P≤0.05) in the flocculating activity of the isolates at different ammonium sulphate concentration. At 72 hrs of incubation, the flocculating activities ranged from 75.44[f] - 83.55[a]% and 77.12[f] - 86.22[a]% respectively. It was discovered that isolate AP4 had its highest flocculating activity at 0.50 g/l of ammonium sulphate concentration while NB2 had its highest at 0.30 g/l.

Fig. 5 shows the effect of varied concentrations of urea on bioflocculant production by AP4 and NB2. There was also a significant difference (P≤0.05) in the flocculating activity of these isolates at different urea concentrations. At 72 hrs of incubation, the flocculating activities ranged from 60.63[e] - 79.22[a]% and 65.51[d] - 83.44[a]% respectively in which the highest flocculating activity of urea for both isolates was observed at 0.70g/L.

This finding contrasts the report of Li et al. [15] on the optimization of nitrogen sources for bioflocculant production using *Bacillus methylotrophicus* C412 strain. The authors observed that urea did not support the growth of the isolate, while 1.0 g/L of beef extract and 3.0 g/L of ammonium sulphate gave the best flocculating activity for the strain. de Koker et al.

[61] reported that high concentration of ammonium sulphate can possibly precipitate protein components in the bioflocculant which could inhibit the extracellular flocculating activity and biomass. Lin and Harichund [62] made use of yeast extract and peptone as the source of nitrogen, and observed that organic nitrogen supported the growth of the isolate than the inorganic form. His view was also supported by Xia et al. [10] and Zheng et al. [11]. Kurane et al. [63]. Lin and Harichund [62] were of the opinion that increase in organic nitrogen sources improved the bioflocculant productions by the bacterial isolates.

3.4 Effect of Dosage Optimization on the Flocculating Activities

Fig. 6 shows the effect of different concentrations of bioflocculants dosage produced by isolates AP4 and NB2 for the flocculation of kaolin solution. There was a significant difference (P≤0.05) in the flocculating activity of the isolates at different dosage concentration. At 72 hrs of incubation, the flocculating activities ranged from 75.78[e] - 86.02[d]% and 76.98[e] - 82.74[a]%, in which the dosage with the highest flocculating activity were 10ml/L and 15ml/L respectively.

This finding was in contrast to the report of Husam and Nisreen [64] on *Azotobacter chrococcum* in which 0.25 mL of bioflocculant dosage was required to obtain the highest flocculating activity of 60.8%. Tang et al. [65] reported that 2.0mg/L of the purified bioflocculant obtained from *Paenibacillus mucilaginosus* gave the best flocculating activity of 98.4%. Maliehe et al. [36] report on the bioflocculants produced by the consortium of *Bacillus pumilus* JX860616 and

Alcaligenes faecalis HCB2 noted that 0.8 mg/ml of the produced bioflocculant gave the best flocculating activity of 85%. Tang et al. [65] observed that an increase in dosage led to decrease in flocculation. This phenomenon was clarified by Yokoi et al. [66] as incomplete dispersion of excess polysaccharide in which only the kaolin particles around the polysaccharides participate in the flocculation reaction. Lee et al. [67] and Kwon et al. [68] explained that the increase in dosage made the excess polysaccharide to be oversaturated on many binding sites of the surface of kaolin particles, as such the force of attraction of the other particles was reduced resulting in a decrease in flocculating activity.

3.5 Application of Bioflocculants Produced by the Selected Strains on Kaolin Solution, Brewery Wastewater and Palm oil Effluent

The Dissolved Oxygen, COD, turbidity, pH, conductivity and salinity of the wastewaters used for this analysis were determined. Table 1 presents the characteristics of the wastewaters.

Table 2 shows the flocculating activities of bioflocculants and Alum in kaolin solution, brewery wastewater and palm oil effluent. It was observed that as the concentration of bioflocculants increases, the flocculating activity of bioflocculants also increased to a certain level.
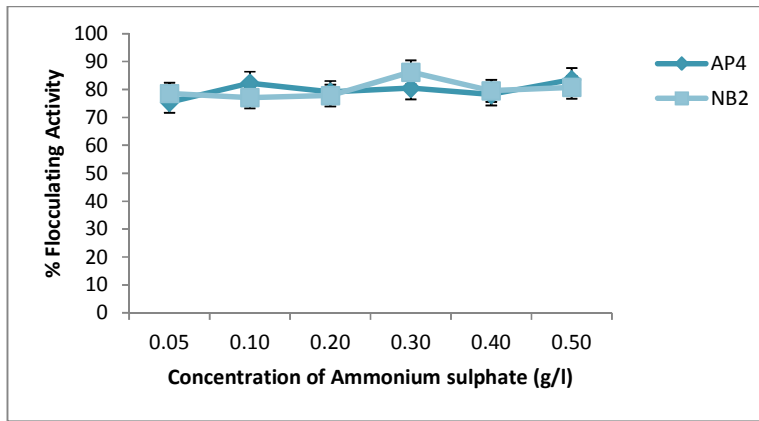

Fig. 4. Effect of different concentrations of ammonium sulphate on the flocculating activities of AP4 and NB2
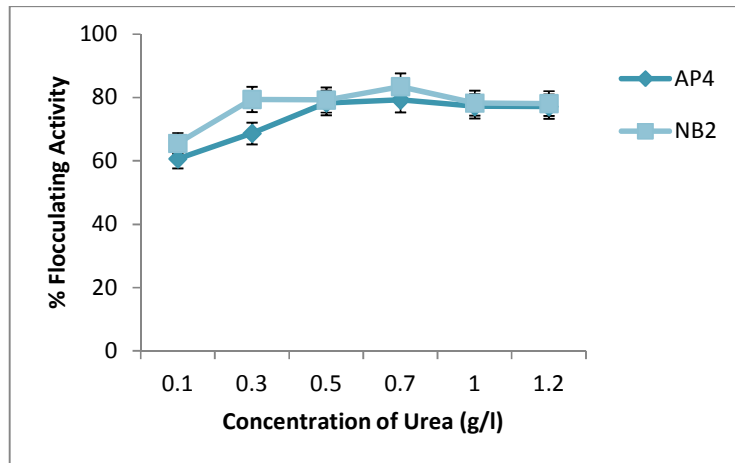

Fig. 5. Effect of different concentrations of urea on the flocculating activities of AP4 and NB2
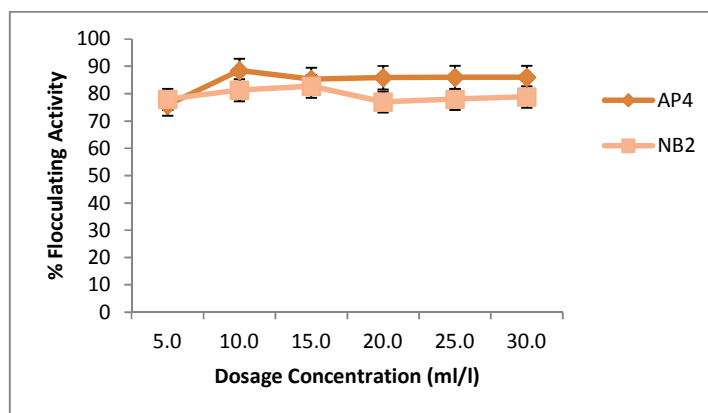
Fig. 6. Effect of dosage on the flocculating activities of AP4 and NB2

Table 1. Characteristics of the wastewaters used for the analysis

Wastewater	Dissolved Oxygen (mg/L)	COD (mg/L)	Turbidity (FTU)	pH	Conductivity (mS/cm)	Salinity (mg/L)
Brewery	9.2 m at 25.4°C	3682	995	9.0	1.22	842
Palm-oil	2.3 at 26.4°C	6988	1530	4.7	0.86	620

Table 2. Flocculating activity of bioflocculants and Alum in kaolin solution

Test materials	Flocculants	% Flocculating activity					
		100 mg/L	200 mg/L	400 mg/L	600 mg/L	800 mg/L	1000 mg/L
Kaolin Solution	AP4	75.78[e]	83.45[d]	88.32[a]	85.93[b]	85.02[c]	85.02[c]
	NB2	77.98[d]	81.31[b]	86.74[a]	78.98[c]	77.98[d]	77.88[d]
	Alum	72.56[e]	76.87[d]	82.61[c]	84.44[a]	86.44[a]	87.32[b]
Brewery Effluent	AP4	76.65[f]	78.11[e]	87.86[b]	89.41[a]	82.78[c]	79.97[d]
	NB2	66.52[e]	75.33[d]	85.81[a]	80.33[c]	80.34[c]	81.02[b]
	Alum	60.67[e]	63.88[d]	71.44[c]	80.56[b]	80.66[b]	82.23[a]
Palm-Oil Effluent	AP4	67.34[f]	70.22[e]	75.44[b]	79.49[a]	74.98[c]	73.01[d]
	NB2	55.22[e]	63.45[d]	70.76[b]	70.88[b]	74.43[a]	68.04[c]
	Alum	50.22[f]	68.33[e]	69.50[d]	72.12[c]	74.44[b]	77.32[a]

Mean followed by different superscript within a column are significantly different (P≤0.05)

At concentration of 400 mg/L, *A. aquatilis* AP4 and *B. clausii* NB2 show the highest flocculating activity of 88.32% and 86.74% respectively in kaolin solution (Table 2) while 600 mg/L was the best concentration required by Alum (84.44%).

Bioflocculants from the two selected strains also showed their ability to flocculate both brewery and palm oil effluent. Concentration of 600 mg/L was required by *A. aquatilis* AP4 as the optimum flocculating dosage with percentage activity of 89.41% while 400 mg/L was needed by *B. clausii* NB2 to produce the optimum flocculating activity (85.81%) in brewery wastewater. Alum had its optimum flocculation of 82.23% at a concentration of 1000 mg/L.

On testing the two bioflocculants on Palm oil effluent, both demonstrated the ability to flocculate the effluent but with a lower percentage of activity when compared with that of kaolin solution and brewery wastewater. This could be as a result of higher turbidity of the palm-oil wastewater compared to that of brewery wastewater (Table 1). In addition, palm oil wastewater contained very high amount of fatty-acids, which could be a factor in the observed flocculation reduction. The concentrations of 600 mg/L and 800 mg/L was needed by *A. aquatilis* AP4 and *B. clausii* NB2 respectively to produce an optimum flocculating activity of 79.49% and 74.43% respectively. For Alum, 1000 mg/L was required to achieve its best flocculating activity of 77.32%.

Lee et al. [69], stated that production industries normally generates different kinds of wastewaters which are made of a wide range of different particles such as organic and inorganic particles, dissolved solids, soluble and insoluble metals, very fine suspended solids, and other impurities. Moreover, the industrial application potential of bioflocculants in the removal of the above mentioned impurities cannot be questioned, as these exo-polysaccharides have been used extensively in the purification of drinking water, treatment of domestic, brewery, pharmaceutical wastewaters, turbidity reduction, BOD, COD, and toxic heavy metals removal, ability to decolorize cationic dyes in wastewaters, harvesting and removal of pathogens of micro-algae from water [24,54,70-71].

There was a significant difference (P≤0.05) in the percentage reduction of the COD and turbidity of the bioflocculants produced by AP4 and NB2 as illustrated in Table 3. In brewery wastewater, AP4, NB2 and Alum were observed to lower the concentration of COD by 87.5, 83.3 and 72.3% respectively, while 78.4, 77.6 and 63.1% reduction were recorded for palm oil effluent. When the bioflocculants were tested for their ability to flocculate highly turbid brewery and palm oil effluents, 88.6, 86.4 and 80.4% reductions in brewery wastewaters were recorded, while, 69.3, 53.7 and 51.5% reduction of palm-oil effluent were observed respectively. According to Choudhary et al. [72], COD is primarily required to oxidize organic matter and is considered as a reference for the organic load of wastewaters, both in the industrial and municipal sectors. Chemical oxygen demand (COD) describes the amount of oxygen that is needed to chemically oxidize organic compounds in water [73]. Bioflocculants produced by both *A. aquatilis*

AP4 and *B. clausii* NB2 demonstrated that they could both flocculate kaolin solution and reduce COD and turbidity level of Brewery wastewater and Palm-oil effluent. Ugbenyen & Okoh [74] and Cosa & Okoh [75] reported that the bioflocculants produced by the consortium of *Oceanobacillus & Halobacillus and the* consortium of *Cobetia & Bacillus sp* were able to reduce the COD of brewery wastewater by up to 99.9 and 95.4% respectively, whereas Gong et al. [4] obtained a lower reduction of 80.4% by a bioflocculant produced by *Serratia ficaria*. However, it should be noted that the initial amount of COD and turbidity of the wastewaters is a very important factor in the determination of reduction percentages.

As shown in Table 3, both AP4 and NB2 bioflocculants were more effective in reducing COD and turbidity of brewery and palm oil wastewaters in comparison to inorganic flocculant (Alum). These results were in accordance with the findings of Ugbenyen and Okoh [74] who revealed that the bioflocculants produced by the consortium of *Cobetia and Bacillus sp.* gave a higher flocculation of kaolin clay of 90%, compared to alum's 66.82%. Moreover, Cosa and Okoh [75] observed that the bioflocculant produced by the consortium of *Oceanobacillus and Halobacillus* gave a better result than both organic and inorganic bioflocculants in reducing COD and turbidity of brewery and dairy wastewaters. Furthermore, Gong et al. [4] demonstrated that bioflocculants produced by *S. ficaria* were able to remove the colour and COD of pulp effluent more effectively than traditional chemical flocculants. Pan et al. [76] obtained 50.6 and 47.5% COD removal rate respectively when bioflocculant PG.a21 Ca and PAC were used to treat domestic wastewater.

Table 3. Effect of bioflocculants on COD and turbidity content of brewery and palm oil wastewater

Flocculant		COD (mg/L)			Turbidity (FTU)		
		Initial	Final	% Reduction	Initial	Final	% Reduction
AP4	Brewery	3682	460	87.5[a]	995	113	88.6[b]
	Palm oil	6988	1509	78.4[c]	1530	470	69.3[e]
NB2	Brewery	3682	615	83.3[b]	995	135	86.4[c]
	Palm oil	6988	1565	77.6[d]	1530	708	53.7[f]
ALUM	Brewery	3682	1020	72.3[e]	995	195	80.4[a]
	Palm oil	6988	2579	63.1[f]	1530	738	51.8[d]

Table 4. Comparison of effectiveness of some common bioflocculants in terms of target wastewater, isolates, COD and turbidity removal percentage, dosage and reaction pH

Applications	Isolate(s)	Bioflocculant	Initial COD (mg/L)	COD removal (%)	Initial turbidity (NTU)	Turbidity removal (%)	Bioflocculant dosage (mg/L)	Reaction pH	Author(s)
Chinese medicine wastewater	*n.r*	F2-F6	n.r	62.6	*n.r*	*n.r*	8, AlCl$_3$, 5*	7.0	[31]
River water	*Serratia ficaria*	S-1	205	87.1	19.0	84.2	4ml/L*	5-7	[4]
Pulp and paper mill effluent	"	S-1	6535	72.1	0.652	98.2	4.0	5-7	[4]
Brewery wastewater,	"	S-1	784	80.7	88.8	91.8	4.0	5-7	[4]
Meat processing wastewater	"	S-1	556	76.3	121.6	93.7	4.0	5-7	[4]
Soy Sauce brewing wastewater	"	S-1	1207	64.1	79.0	93.7	4.0	5-7	[4]
Domestic wastewater	*n.r*	PG.a21 Ca	143	50.6	*n.r*	*n.r*	*n.r*	*n.r*	[76]
Swine wastewater	*n.r*	xn11 + xn7	1372-3025	42%	230-800	91,	50-80 *	8.0	[14]
Brewery Wastewater	Consortium of *Oceanobacillus & Halobacillus*	*n.r*	8213	99.7	750	93.9	0.2	5.62	[75]
Dairy wastewater	"	*n.r*	4813	99.9	1382	88.3	0.2	7.5	[75]
River water	"	*n.r*	92	70.8	174	98.6	0.2	7.2	[75]
Livestock wastewater	*Rhodococcus erythropolis*	RSF	1,400 – 1,600	63	170–220	93.9	40; CuSO$_4$ 80	11	[26]
Brewery wastewater	Consortium of *Cobetia & Bacillus sp*	-	821	95.4	750	94.8	*n.r*	8.0	[74]
Dairy Wastewater	"	-	4813	99.1	1382	81.2	*n.r*	8.0	[74]
River water	"	-	92	73.9	174	98.9	*n.r*	8.0	[74]
Algae-laden lake water	"	-	132	72.0	18.2	93.4	*n.r*	8.0	[74]
Starch wastewater,	*Consortium of R. radiobacter & B.*	CBF	*n.r*	88	*n.r*	*n.r*	14; CaCl$_2$, 1.5*	7.5	[15]

Applications	Isolate(s)	Bioflocculant	Initial COD (mg/L)	COD removal (%)	Initial turbidity (NTU)	Turbidity removal (%)	Bioflocculant dosage (mg/L)	Reaction pH	Author(s)
	sphaeicus								
Printing& dyeing wastewater	"	CBF	*n.r*	66	*n.r*	*n.r*	14; CaCl$_2$, 1.5*	7.5	[15]
Landfill leachate	"	CBF	*n.r*	58	*n.r*	*n.r*	14; CaCl$_2$, 1.5*	7.5	[15]
Aquaculture wastewater	*B. megaterium*	SP1	35.6	64	27.1	83.7	*n.r*	7.0	[50]
Dairy wastewater	*B. subtilis*	-	45600**	43.8	50.50**	19.6	n.r	6.5	[77]
Domestic wastewater	*n.r*	-	317	89.7%	83	91.8%	40.0	7.5	[19]
Brewery wastewater	*B. clausii*	AP4	3682	87.5	995	88.6	10 & 1% CaCl$_2$	7.0	Present study
Brewery wastewater	*A. aquatilis*	NB2	3682	83.3	995	86.4	10 & 1% CaCl$_2$	7.0	Present study
Palm oil wastewater	*B. clausii*	AP4	6988	78.4	1530	69.3	10 & 1% CaCl$_2$	7.0	Present study
Palm oil wastewater	*A. aquatilis*	NB2	6988	77.6	1530	53.7	10 & 1% CaCl$_2$	7.0	Present study

n.r means not reported.
* reported in ml/L
** reported in mg/L

4. CONCLUSION

The effect of optimizing carbon and nitrogen sources as well as dosage of bioflocculants produced by *A. aquatilis* AP4 and *B. clausii* NB2 were studied. 15.0, 0.50, 0.70 g/L and 10 ml/L of glucose, ammonium sulphate, urea and dosage respectively were required by AP4 to have the best flocculating activity in kaolin solution, while 20.0, 0.30, 0.70 g/L and 10 ml/L of glucose, ammonium sulphate, urea and dosage were required by NB2. The consortium of the two isolates had a significant influence (p≤0.05). The bioflocculants were able to flocculate kaolin solution, brewery wastewater and palm-oil effluent at a lower dosage and effectively reduce the COD and turbidity of wastewaters.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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