

Original Research Article

EFFECT OF OPTIMIZATION OF CULTURAL PARAMETERS ON EXOBIOPOLYMER PRODUCTION BY MICROBIAL ISOLATES AND THEIR APPLICATION IN WASTEWATERS TREATMENT

ABSTRACT

The importance of optimization of culture media and conditions in obtaining effective, efficient and optimum production of exopolymers by microorganisms cannot be overemphasized. As such, this work aimed to investigate the effect of optimizing culture media on the bioflocculant production. It also studied the application of bioflocculants in the treatment of wastewaters. *Alcaligenes aquatilis* AP4 and *Bacillus clausii* NB2 obtained from wastewaters samples were the two isolates used for this study. The basal medium used for the batch fermentation was supplemented with different concentration of carbon and nitrogen sources. The produced bioflocculants were tested on Kaolin solution, Brewery and palm-oil effluents in concentration ranging from 10-1000ppm. It was observed that optimization of culture media by the two isolates, had a significant effect ($P \leq 0.05$) on the bioflocculant production by the strains. At 72 hrs of incubation, 15.0 and 20.0g/l glucose concentration gave the best conditions for bioflocculant production by isolates AP4 and NB2 respectively. Inorganic nitrogen source $((\text{NH}_4)_2\text{SO}_4)$ required 0.50 and 0.30 g/l concentration while the organic nitrogen source (Urea) required 0.70g/L concentration for both isolates. 10 and 15ml/L of dosage concentrations respectively gave the best results. Moreover, the ability of the bioflocculants to flocculate kaolin solution, brewery wastewater and palm-oil effluent were confirmed. In addition, the bioflocculants also showed a tremendous abilities to reduce COD and turbidity of wastewaters.

Keywords: bioflocculant, biopolymer, optimization, COD, turbidity, flocculating activity.

INTRODUCTION

Biopolymers could be grouped into either intracellular or extracellular (Nwodo *et al.*, 2012). 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 (Vijayabaskar *et al.*, 2011). Some of these microbes synthesize their biopolymer in form of bioflocculant which could be polysaccharides (Yim *et al.*, 2007; Gong *et al.*, 2008; Lian *et al.*, 2008; Liu *et al.*, 2010), glycoprotein (Okaiyeto *et al.*, 2013; Abdel-Aziz *et al.*, 2015; Ogunsade *et al.*, 2015) or functional proteins (Xia *et al.*, 2008; Zheng *et al.*, 2008).

These bioflocculant producing organisms are generally present in environmental samples such as soil (Arafa *et al.*, 2014; Jiang *et al.*, 2015), activated sludge (Zhang *et al.*, 2012; Li *et al.*, 2015), crude oil (Zaki *et al.*, 2011) wastewaters (Dlangamandla *et al.*, 2016; Nurul-Adela *et al.*, 2016), and lignocellulosic biomass (Guo *et al.*, 2016). Generally, microbial flocculants can be produced by eubacteria, actinomycetes and fungi during their growth and it is preferred to the conventional inorganic and organic flocculants because of their biodegradability, harmless to humans and the environment, safety for ecosystems, high flocculating capability and lack secondary pollution (Gao *et al.*, 2006; Azmi *et al.*, 2015). This bioflocculant can be used in industrial and wastewaters treatment processes such as pharmaceutical, fermentation, food industries dredging and downstream processing (Seo, 1993; Zhang *et al.*, 1999; Salehizadeh and Shojaosadati, 2001).

Specifically, bioflocculants have been applied in various ways and found to be potent in the removal of heavy metal ions, turbidity and COD from effluents (Zhang *et al.*, 2012; Zhao *et al.*, 2017), removal of dye and precipitation of inorganic solid particles from suspension (Bender *et al.*, 1994; Peng *et al.*, 2014), treatment of drinking water (Zhang *et al.*, 2015), treatment of oil-polluted wastewaters and landfill leachate (Liu *et al.*, 2012; Li *et al.*, 2015).

Moreover, the high cost of bioflocculant has been one of the limiting factors towards its application (Zhang *et al.*, 2010), as such, different culture media have been experimented as substrates for bioflocculant production to cushion this effect such as dairy and sauce wastewaters, effluent from a hydrogen-producing bioreactor (Zhou *et al.*, 2003; Li *et al.*, 2004; Dong *et al.*, 2008); Brewery, Palm-oil and Sewage effluents (Adebayo-tayo and Adebami, 2014); lignocellulosic biomass (Liu *et al.*, 2017), soybean Juice and fishmeal wastewaters (Huang *et al.*, 2001; Zhou *et al.*, 2003). Alternatively, optimization of culture conditions such as carbon and nitrogen sources, bioflocculant dosage concentration, pH and temperature are important to obtain effective and optimum bioflocculants. Effective optimization will also reduce the problem created by high cost of production. As such, this research work is carried out to determine the effect of optimizing the concentration of culture parameters on the bioflocculants production and the application of the produced bioflocculants in the treatment of wastewater samples.

MATERIAL AND METHODS

Culture preparation

Isolation and characterization of bioflocculants producing isolates AP4 and NB2 obtained from palm oil mill effluent and brewery wastewater respectively in south-western part of Nigeria had been previously reported. The strains were preserved in 20% glycerol stock at -80°C in the Department of Microbiology University of Ibadan, Ibadan Nigeria. 16SrDNA Sequence determination and Phylogenetic Analysis of the two strains showed that AP4 and NB2 were *A. aquatilis* and *B. clausii* respectively. The FTIR and chemical analysis conducted on the two isolates revealed them to be glycoproteins.

The stock cultures were re-isolated and maintained on Nutrient agar, incubated at 30°C for 72h and stored at 4°C. The seed culture was grown in a 250ml flask containing: Nutrient broth, 10.0g; Potato dextrose broth, 5.0g; Glycerol, 3ml; Yeast Extract, 6.5g; Sodium chloride, 1.0g in 1litre 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 24hrs.

Bioflocculant production

The isolates were used for bioflocculant production using Bioflocculant Production Broth medium (BPB). The BPB composition include: 10g glucose, 2g KH₂PO₄, 0.2g MgSO₄.7H₂O, 0.1NaCl, 0.5g CaCO₃, and 0.5g 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 5,000mg/l were used to evaluate the flocculating abilities of the produced exopolysaccharides (Zheng *et al.*, 2008).

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. 1m of each strain were then inoculated into a fresh 250ml Erlenmeyer flask containing culture broth. The medium was incubated at 25°C on a rotary shaker at 120rpm 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 5,000mg/l. The optical density (OD) of the clarifying upper phase solution was measured at 550nm with a UV spectrophotometer (Maliehe *et al.*, 2016).

Determination of Flocculating Activity

The flocculating activity was determined according to the method of Kurane *et al.* (1986) as modified by Gao *et al.* (2006). 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% CaCl₂ (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 10mL 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:

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

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

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 (120rpm) condition. After incubation the Kaolin assay was carried out to check the maximum flocculating activity of the isolates after which the absorbance was then read using a spectrophotometer at a wavelength of 550nm.

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 Omi-asoro, Ilesha, Osun State while Palm-oil effluent was collected from Fiditi village, Oyo State. The Dissolved Oxygen (DO), Chemical dissolved oxygen (COD), Turbidity, pH, Conductivity, Salinity and Temperature of the wastewater samples were determined. All wastewaters were stored at 4°C before flocculation. Varied concentrations (100-1000ppm) i.e. (0.1-1.0ml/l) of bioflocculant and 1 ml of 1% CaCl₂ 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 550nm 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.* (2008). The results obtained were compared with chemically synthesized flocculants in which the bioflocculant was replaced with Aluminium sulphate (Alum).

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 (Kao and Green, 2008).

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 Figure 1.

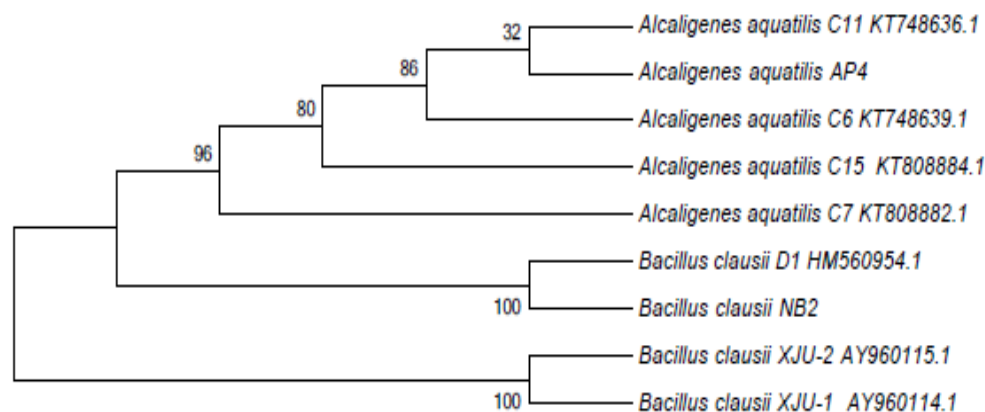
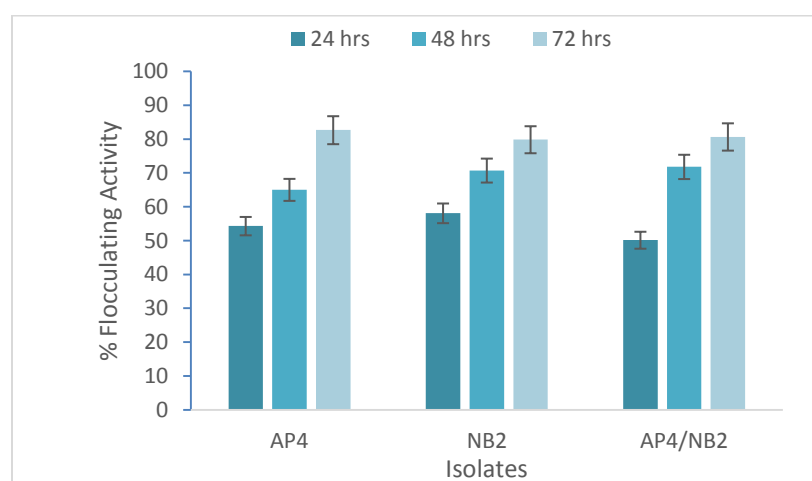


Figure 1: Phylogenetic tree showing the relationships between *A. aquatilis* AP4 as well as *B. clausii* NB2 strains and other closely related species collected from the Gene Bank. The dendrogram was generated by the neighbor-joining method. Bootstrap values per 100 bootstrap analysis presented for values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

Effect of single starter/consortium on biofloculant production by the isolates

The production of biofloculant and its activity by single starter and consortium isolates are shown in Figure 2. It was observed that there was a significant difference ($P \leq 0.05$) in the flocculating activity of 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. Recently, there has been growing interest in the biotechnological consortia synthesis of isolates for 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 (Zuroff and Curtis, 2012). For instance, Microbial consortium have been earmarked as a reliable and efficient alternative to single strains for lignocellulose degradation (Brossi *et al.*, 2015). Cortes-Tolalpa *et al.* (2016) 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 (Mee and Wang, 2012). Maliehe *et al.* (2016) observed an increased yield when

the consortium of *B. pumilus* JX860616 and *A. faecalis* HCB2 were used for bioflocculant production. Ntsaluba *et al.* (2013) reported 89% flocculating activity for the consortium of *Methylobacterium* sp. Obi and *Actinobacterium* sp. Mayor. Menetrez, (2012) and Wang *et al.* (2016) reported that a lower activity of consortium may be sometimes noticed because synthetic microbial consortia are often fragile and unstable and as such limit their applications in the industrial biotechnology level which may be in contrast to what is obtained in their environment. Kim *et al.* (2008) 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.



188

189 **Figure 2:** Effect of single and combined starter on the bioflocculation productions

190 Key: AP4= *A. aquatilis* AP4; NB2= *B. clausii* NB2 and AP4/NB2= Consortium by *A. aquatilis* AP4 and *B. clausii* NB2

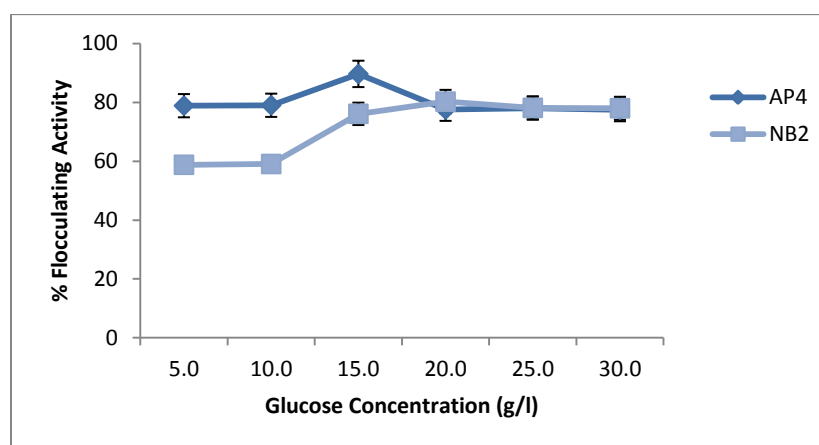
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192 ***Effect of glucose optimization on the flocculating activities of the isolates***

193 Figure 3 shows the effect of varied concentrations of glucose on bioflocculant production by
 194 AP4 and NB2. At 72 hrs of incubation, there was a significant difference ($P \leq 0.05$) in the
 195 flocculating activity of different glucose concentration, which ranged from 77.44^f - 89.66^a % and
 196 58.79^f - 80.23^a % for isolates AP4 and NB2 respectively. It was discovered that isolate AP4 had
 197 its highest flocculating activity at 15.0 g/l of glucose concentration while NB2 had its highest at
 198 20.0g/l.

199 Carbon source is the backbone of functional biological molecules which could be
 200 polysaccharides, disaccharides, monosaccharides and alcohol sugar (Minty *et al.*, 2013; Wang *et al.*,
 201 2016). Glucose is a monosaccharide which when added to nutrient broth may increase the
 202 overall growth rates and biomass of bacteria over time (Cosa *et al.*, 2013). Moreover, there have
 203 been various reports of complex sugars being used for bioflocculant production. For instance, Li
 204 *et al* (2017) reported that starch was found the most favourable by *Oceanob. polygoni* in the

205 production of bioflocculant MBF-HG6. Alternatively, glucose may be inhibitory to cell growth if
 206 the concentration is too high (Dalhoff, 1979; Borralho *et al.*, 2002; Schmidt-Glenewinkel and
 207 Barkai, 2014). Increasing the amount of glucose have been shown to limit microbial growth by
 208 inhibiting proteinaceous enzymes (Boyd and Lichstein, 1951; Wang *et al.*, 2015). This result was
 209 in accordance with Luo *et al.* (2016) that 25g/l of glucose concentration was required by
 210 bioflocculant-producing *Bacillus megaterium* SP1 to obtained 90.6% flocculating activity. Liu *et*
 211 *al.* (2016) reports on alkali-tolerant strain of *Microbacterium esteraromaticum* C26 showed that
 212 glucose gave the best among the carbon sources tested at concentration 3g/L which is in contrast
 213 to the present study. A similar finding was reported by Okeiyato *et al.* (2016a) that 20g/l of
 214 glucose was required by *Bacillus*. sp. AEMREG7 to obtain the best flocculating activity of
 215 88.03% among several carbon sources tested.



216
 217 **Figure 3:** Effect of different conc. of glucose on the flocculating activities of AP4 and NB2

218 **Effect of optimization of Nitrogen sources on the flocculating activities of the isolates**

219 Different concentration of organic (Urea) and inorganic (Ammonium sulphate) nitrogen sources
 220 on the flocculating ability of the isolates were investigated. Figure 4 shows the effect of varied
 221 concentrations of Ammonium sulphate on bioflocculant production by AP4 and NB2. There was
 222 a significant difference ($P \leq 0.05$) in the flocculating activity of the isolates at different
 223 Ammonium sulphate concentration. At 72 hrs of incubation, the flocculating activities ranged
 224 from 75.44^f - 83.55^a % and 77.12^f - 86.22^a % respectively. It was discovered that isolate AP4 had
 225 its highest flocculating activity at 0.50 g/l of Ammonium sulphate concentration while NB2 had
 226 its highest at 0.30 g/l.

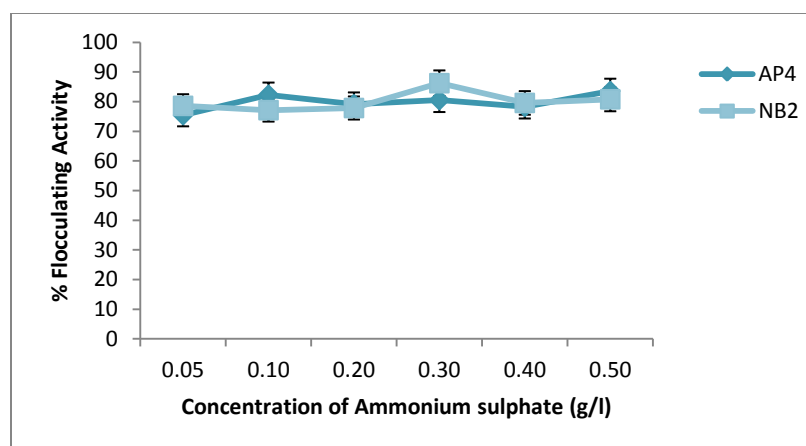


Figure 4: Effect of different conc. of Ammonium sulphate on the flocculating activities of AP4 and NB2

Figure 5 shows the effect of varied concentrations of urea on bioflocculant production by AP4 and NB2. There was also a significant difference ($P \leq 0.05$) 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.

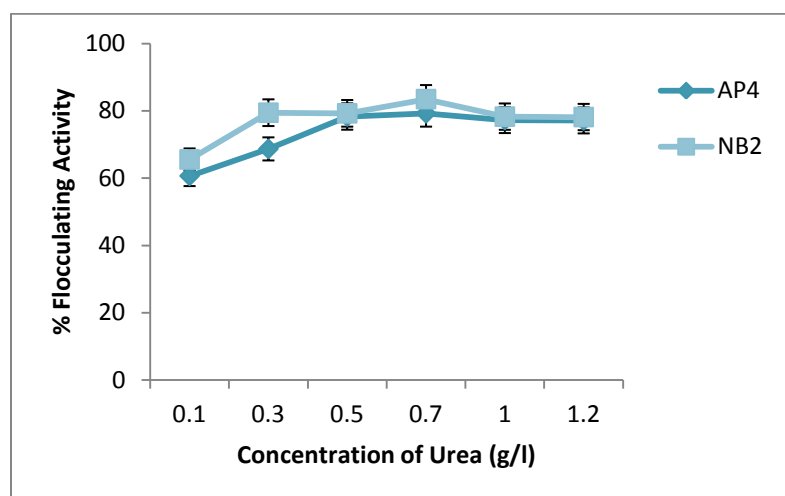


Figure 5: Effect of different conc. of Urea on the flocculating activities of AP4 and NB2

According to the research carried out by Li *et al.* (2015) on the optimization of nitrogen sources on *Bacillus methylotrophicus* C412 strain, Urea did not support the growth of the isolate, while, 1.0g/L of beef extract and 3.0g/L of ammonium sulphate give the best flocculating activity for the strain. It was also noted that high concentration of ammonium sulphate can possibly precipitate protein components in the bioflocculant which could inhibit the extracellular flocculating activity and biomass (de Koker *et al.*, 2004). Lin and Harichund, (2012) 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 in-organic. His view was also supported by Xia *et al.* (2008) and Zheng *et al.* (2008). Kurane *et al.* (1994) and Lin and Harichund (2012) were of the opinion that increase in organic nitrogen sources improved the biofloculant productions by the bacterial isolates.

Effect of dosage optimization on the flocculating activities

Figure 6 shows the effect different concentration of biofloculants dosage produced by isolates AP4 and NB2 for the flocculation of kaolin solution. There was a significant difference ($P \leq 0.05$) 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 % and in which the highest flocculating activity of dosage was 10ml/L and 15ml/L respectively.

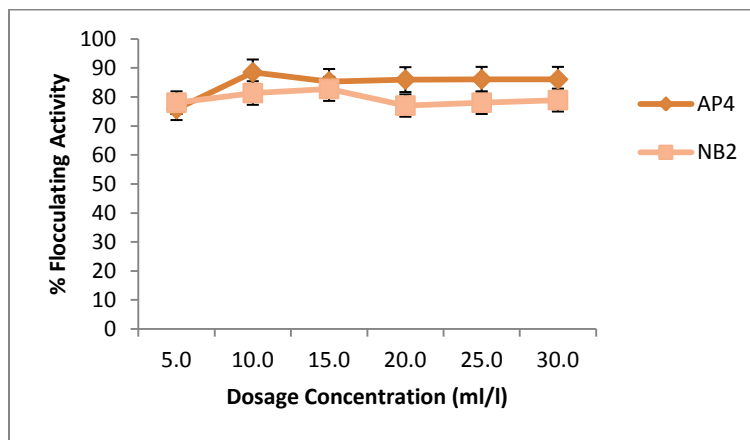


Figure 6: Effect of dosage conc. on the flocculating activities of AP4 and NB2

Husam and Nisreen, (2013) on *Azotobacter chroococcum* reported that 0.25 mL of biofloculant dosage was required to obtain the highest flocculating activity of 60.8%, an increase in dosage lead to decrease in flocculation. 0.1mg/mL and 2.0mg/L of the biofloculant produced by *Bacillus pumilus* and *Paenibacillus mucilaginosus* was required to obtain the best flocculating activity of 96.5 and 97% respectively. Yokoi *et al.* (1997) explained the phenomenon as incomplete dispersion of excess polysaccharide in which only the kaolin particles around the polysaccharides participated in the flocculation reaction. Lee *et al.* (1995) and Kwon *et al.* (1996) 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 which made the flocculating activity to decrease.

Application of biofloculants produced by the selected strains on Kaolin solution, Brewery wastewater and Palm-oil effluent

The amount of dissolved oxygen, COD, Turbidity, pH, conductivity and salinity of the wastewaters used for this analysis were determined. Table 1 showed the characteristics of the wastewaters.

Table 1: Characteristics of the wastewaters used for the analysis

Wastewater	Dissolve Oxygen (mg/L)	COD (mg/L)	Turbidity (FTU)	pH	Conductivity (S/cm)	Salinity (ppm)
Brewery	9.2m 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 shows the flocculating activities of biofloculants and Alum in Kaolin solution, Brewery wastewater and Palm-oil effluent. It was observed that as the concentration of biofloculants increases, the flocculating activity of biofloculants also increases to a certain level. At concentration of 400ppm, *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 600ppm was the best concentration required by Alum (84.44%).

Table 2: Flocculating activity of biofloculants and Alum in Kaolin solution

Test Materials		% Flocculating Activity					
		100 ppm	200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm
KAOLIN	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 ($P \leq 0.05$).

Biofloculants from the two selected strains also show their ability to flocculate both brewery wastewater and palm-oil effluent. Concentration of 600ppm was required by *A. aquatilis* AP4 with percentage activity of 89.41% while 400ppm was needed by *B. clausii* NB2 to produced the best flocculating activity (85.81%) in Brewery wastewater. Alum has its best flocculation of 82.23% at a concentration of 1000ppm.

On testing the two bioflocculants on Palm-oil effluent, it was observed that both showed the ability to flocculate this effluent but with lower percentage of activity when compared with that of Kaolin solution and Brewery wastewater. This is 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 concentration of 600ppm was needed by *A. aquatilis* AP4 to produce best flocculating activity of 79.49% and 800ppm by *B. clausii* NB2 to carry out the same task (74.43%), whereas 1000ppm was required by Alum to produce its best flocculating activity of 77.32%.

Lee *et al.* (2014), 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 (Lei *et al.*, 2015; Okeiyato *et al.*, 2016b; Li *et al.*, 2017; Zhao *et al.*, 2017).

Table 3: Effect of bioflocculants on COD and turbidity content of brewery and palm-oil wastewaters

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

There was a significant difference ($P \leq 0.05$) 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.* (2011), 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 oxidizes organic compounds in water, using an environmentally oxidant (Ajayi *et al.*, 2016). Biofloculants produced by both *A. aquatilis* AP4 and *B. clausii* NB2 demonstrated that they could both flocculate Kaolin solution and reduced COD and turbidity level of Brewery wastewater and Palm-oil effluent. Ugbenyen and Okoh, (2014) and Cosa & Okoh, (2014) reported that the biofloculants produced by the consortium of *Oceanobacillus* & *Halobacillus* and the consortium of *Cobetia* & *Bacillus* *sp* were able to cause reduction in the COD of brewery wastewater up to 99.9 and 95.4% respectively, whereas Gong *et al.* (2008), obtained a lower reduction of 80.4% by a biofloculant 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. Li *et al.* (2017) observed 91.08% reduction in turbidity of tannery wastewater by the biofloculant produced by salt-tolerant alkaliphilic *Oceanobacillus polygoni*.

As shown in table 3, both AP4 and NB2 biofloculants were more effective in reducing COD and turbidity of brewery and palm-oil wastewaters in comparison to inorganic flocculant (Alum). These results are in accordance with the finding of Ugbenyen and Okoh, 2014 who revealed that the biofloculants produced by the consortium of *Cobetia* & *Bacillus* *sp.* gave a higher flocculation of kaolin clay of 90%, compared to alum's 66.82%. Moreover Cosa and Okoh, (2014) observed that the biofloculant produced by the consortium of *Oceanobacillus* & *Halobacillus* gave a better result than both organic and inorganic biofloculants in reducing COD and turbidity of brewery and dairy wastewaters. Furthermore, Gong *et al.* (2008) demonstrated that biofloculants produced by *S. ficaria* were able to remove the colour and chemical oxygen demand (COD) of pulp effluent more effectively than traditional chemical flocculants. Pan *et al.* (2009) obtained 50.6 and 47.5% COD removal rate respectively when biofloculant PG.a21 Ca and PAC were used to treat domestic wastewater.

Table 4: Biofloculant applications, isolates, COD and turbidity removal percentage, dosage and reaction pH of some researched biofloculants

Applications	Isolate(s)	Biofloculant	Initial COD (mg/L)	COD removal (%)	Initial turbidity (NTU)	Turbidity removal (%)	Biofloculant Dosage (mg/L)	Reaction pH	Author(s)
Chinese medicine wastewater	<i>n.r</i>	F2-F6	<i>n.r</i>	62.6	<i>n.r</i>	<i>n.r</i>	8, AlCl ₃ , 5*	7.0	Dong <i>et al.</i> , 2008
River water	<i>Serratia ficaria</i>	S-1	205	87.1	19.0	84.2	4ml/L*	5-7	Gong <i>et al.</i> , 2008
Pulp and paper mill effluent	"	S-1	6535	72.1	0.652	98.2	4.0	5-7	"
Brewery wastewater,	"	S-1	784	80.7	88.8	91.8	4.0	5-7	"
Meat processing wastewater	"	S-1	556	76.3	121.6	93.7	4.0	5-7	"
Soy Sauce brewing wastewater	"	S-1	1207	64.1	79.0	93.7	4.0	5-7	"
Domestic wastewater	<i>n.r</i>	PG.a21 Ca	143	50.6	<i>n.r</i>	<i>n.r</i>	<i>n.r</i>	<i>n.r</i>	Pan <i>et al.</i> , 2009

Swine wastewater	<i>n.r</i>	xn11 + xn7	1372- 3025	42%	230-800	91,	50-80 *	8.0	Zhang <i>et al.</i> , 2012
Oily wastewater	<i>Proteus vulgaris</i>	KL3-5.	1036.5	30	237.2	61*	0.05	7.4	Liu <i>et al.</i> , 2012
Brewery Wastewater	Consortium of <i>Oceanobacillus</i> & <i>Halobacillus</i>	<i>n.r</i>	8213	99.7	750	93.9	0.2	5.62	Cosa & Okoh, 2014
Dairy Wastewater	"	<i>n.r</i>	4813	99.9	1382	88.3	0.2	7.5	"
River Water	"	<i>n.r</i>	92	70.8	174	98.6	0.2	7.2	"
Livestock wastewater	<i>Rhodococcus erythropolis</i>	RSF	1,400 – 1,600	63	170–220	93.9	40; CuSO ₄ 80	11	Peng <i>et al.</i> , 2014
Brewery Wastewater	Consortium of <i>Cobetia</i> & <i>Bacillus sp</i>	-	821	95.4	750	94.8	<i>n.r</i>	8.0	Ugbenyen and Okoh, 2014
Dairy Wastewater	"	-	4813	99.1	1382	81.2	<i>n.r</i>	8.0	"
River Water	"	-	92	73.9	174	98.9	<i>n.r</i>	8.0	"
Algae-laden lake water	"	-	132	72.0	18.2	93.4	<i>n.r</i>	8.0	"
Starch wastewater,	Consortium of <i>R. radiobacter</i> & <i>B. sphaericus</i>	CBF	<i>n.r</i>	88	<i>n.r</i>	<i>n.r</i>	14; CaCl ₂ , 1.5*	7.5	Li <i>et al.</i> , 2015
Printing& dyeing wastewater	"	CBF	<i>n.r</i>	66	<i>n.r</i>	<i>n.r</i>	14; CaCl ₂ , 1.5*	7.5	"
Landfill leachate	"	CBF	<i>n.r</i>	58	<i>n.r</i>	<i>n.r</i>	14; CaCl ₂ , 1.5*	7.5	"
Aquaculture wastewater	<i>B. megaterium</i>	SP1	35.6	64	27.1	83.7	<i>n.r</i>	7.0	Luo <i>et al.</i> , 2016
Dairy wastewater	<i>B. subtilis</i>	-	45600**	43.8	50.50**	19.6	<i>n.r</i>		Didar& Ferdosi- Makan, 2016
Domestic wastewater	<i>n.r</i>	-	317	89.7%	83	91.8%	40.0	7.5	Guo <i>et al.</i> , 2016
Brewery wastewater	<i>B. clausii</i>	AP4	3682	87.5	995	88.6	10 & 1% CaCl ₂	7.0	Present study
Brewery wastewater	<i>A. aquatilis</i>	NB2	3682	83.3	995	86.4	10 & 1% CaCl ₂	7.0	Present study
Palm oil wastewater	<i>B. clausii</i>	AP4	6988	78.4	1530	69.3	10 & 1% CaCl ₂	7.0	Present study
Palm oil wastewater	<i>A. aquatilis</i>	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 ppm

CONCLUSION

Optimization of Carbon and Nitrogen sources as well as dosage of biofloculants producing *A. aquatilis* AP4 and *B. clausii* NB2 were done. 15.0, 0.50, 0.70g/L and 10ml/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, 0.70g/L and 10ml/L of glucose, ammonium sulphate, urea and dosage were required by NB2. The consortium of the two isolates have a significant influence ($p \leq 0.05$). The biofloculants 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.

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