Comparative effect of medium composition on bioflocculant production by microorganisms isolated from wastewater samples

Gboyega Adebami, Adebayo-Tayo Bukola Christianah.

Department of Microbiology, University of Ibadan, Ibadan, Oyo state, Nigeria
Corresponding Author: bukola.tayo@gmail.com

Abstract: A bioflocculant is a kind of biodegradable polymeric flocculants produced by many microorganisms including bacteria, fungi and actinomycetes during their growth. Bioflocculant have special advantages such as safety for ecosystems, potential flocculating effects, biodegradability and harmlessness to humans and the environment. Based on these unique properties it may potentially be applied in production industries for various purposes. In this study, a total of thirty nine (39) bacteria were isolated from three different wastewater samples from Oyo and Osun states. The isolates were screened for bioflocculant production using kaolin solution (5g/L) as a test material. There was a significant different (P≥0.05) in the flocculating ability of the isolates. The percentage flocculating activity ranged from 03.00^{p} – 75.63^{a} % in which isolate TB3 had the highest followed in order by isolates TM3, AM6 and API, while isolate AP3 had the least. Six isolates with the best flocculating activity selected and identified phenotypically were: *Enterobacter asburiae* AM6, *Streptococcus plurextorum* AP1, *Solibacillus silvestri* AP4, *Bacillus licheniformis* NB2, *Citrobacter* sp. TB3 and *Paenibacillus polymyxa* TM3. The isolates were used for bioflocculant production using different medium (Medium 1, 2, 3 and 4 respectively). There was a significant difference (P≥0.05) in the flocculating activity of the isolates in production medium. In Medium 1 the percentage flocculating activity ranged from 31.0 -89.19% in which isolate AP4 had the highest. About 33.5% of the isolate (AP4 and TB2) had flocculating activity above 70%. In Medium 2 the percentage flocculating activity ranged from 32.91 -89.19% in which isolate AP4 had the highest. About 83.3% of the isolate (AM6, AP1, AP4, NB2 and TB2) had flocculating activity above 70%. In Medium 3 the percentage flocculating activity ranged from 34.81 -78.38% in which isolate AP4 had the highest. About 66.7% of the isolate (AM6, AP1, AP4 and TB2) had flocculating activity above 70%. In Medium 4 the percentage flocculating activity ranged from 34.65 -88.02% in which isolate NB2 had the highest. About 66.7% of the isolate (AM6, AP1, AP4 and NB2) had flocculating activity above 70%. [Gboyega Adebami. Adebayo-Tayo Bukola Christianah. **Comparative effect of medium composition on bioflocculant production by microorganisms isolated from wastewater samples.** *Rep Opinion* 2013;5(2):46-53]. (ISSN: 1553-9873). http://www.sciencepub.net/report. 12

Key words: Waste waters, Bacteria, Bioflocculant, Production medium, Flocculating activity.

1. Introduction

Flocculants are used for the aggregation of colloidal substances and cellular materials and thus are widely applied in different industrial processes, including wastewater treatment, downstream processing, food and fermentation processes (Nakata and Kurane, 1999). Although the words "coagulation" and "flocculation" are often used interchangeably, they refer to two distinct processes (Sundstrom and Klei, 1979). Coagulation indicates the process through which colloidal particles and very fine solid suspensions are destabilized so that they can begin to agglomerate if the conditions are appropriate· Flocculation refers to the process by which destabilized particles actually conglomerate into larger aggregates so that they can be separated from the wastewater (Droste, 1997). Inorganic flocculating agents, such as aluminum sulfate, polyaluminum chloride, ferric chloride and organic polymers such as polyacrylamide derivatives, are frequently used in both wastewater treatment and the fermentation industries because they are not only cost-effective, but also have strong flocculating activity (He *et al*., 2002). However, studies have shown that some of the chemically synthetic flocculating substances are not only harmful to both humans and the environment, but are also non-degradable in nature (Taniguchi *et al*., 2005).

The use of bioflocculants in wastewater treatment seems to be an economical alternative to physical and chemical means (Vijayalakshmi and Raichur, 2003). Bioflocculants are capable of removing inorganic/ organic particles through their flocculating activity. It has been investigated that bioflocculant is effective in removing suspended solids, heavy metals and bacteria, and in reducing the turbidity of different types of industrial wastewater effluents (Kurane *et al*., 1994; Gao *et al*., 2009; Lin and Harichund, 2011).

Knowledge of microbial growth and substrate utilization has been of tremendous help for the prediction of the fate of organic compounds in natural and engineered environments (Grady *et al*., 1996). Bacteria generally have distinct properties when starved for each class of essential nutrients. The

related depletions require completely different physiological and regulatory responses (Ferenci, 1999, 2001). In the natural environment, just as in different culture media, heterotrophic bacteria adapt their growth rate and cell size to both the nature and concentrations of organic nutrients (Eguchi *et al.,* 1996).

It has been observed that some variations existed in the media composition used by various researchers as bioflocculant production medium. This study therefore investigated the important of media compositions for the growth of bioflocculant producing microorganisms and the ability of the microbes to produce the expected exopolysaccharides in the require quantity.

2. Material and Methods

2.1 Sample collection

Samples were collected from three different wastewater sources in western part of Nigeria; which are Brewery effluent (from International Brewery Plc, Omiasoro, Ilesa, Osun State); Palm oil effluent and Abattoir effluent (from Ibadan in Oyo State). The samples were collected using sterile containers and transported to the laboratory for further analysis.

2.2 Isolation of associated microorganisms from the samples

Isolation of microorganisms associated with the effluents was carried out using serial dilution technique. 9ml of the effluents was introduced into 90ml of sterile distilled water to make up one in ten dilutions which were further diluted up to 10^{-8} dilution from 10^{-4}, 10^{-6} and 10^{-8} dilution. 1ml of each diluents was pipette into sterile plates and then mixed with molten Tryptone Soy Agar (TSA) for the isolation of *Bacillus* strains, Actinomyces agar (a compounded medium for Actinomyces with the following composition: Nutrient agar - 10.0g; PDA/SDA - 5.0g; Glycerol - 3ml; Yeast Extract - 6.5g; Sodium chloride - 1.0g in 1litre of distilled water) and Nutrient agar for the isolation of Actinomyces and heterotrophs. The plates were thereafter gently swirled, allowed to set while still on the working bench. The plates were incubated at 37^0C for 24hrs.

2.3 Screening of the isolates for bioflocculant production

The isolates were screened for bioflocculant production using Bioflocculant Production Broth medium (BPB). The BPB composition include: 10g glucose, 2g KH_2PO_4, 0.2g $MgSO_4.7H_2O$, 0.1NaCl, 0.5g $CaCO_3$, and 0.5g yeast extract. The mixture was dissolved in 1 liter de-ionized water with the initial pH adjusted to 7.0. The medium was sterilized, inoculated with pure culture of the isolate 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 capability of a series of the culture broths.

2.4 Determination of Flocculating Activity

The flocculating activity was determined according to the method of Kurane *et al*., (1994) 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 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 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:

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

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

2.5 Selection of bioflocculant producing strains

After the determination of flocculating activity of the isolates, six isolates which produced the highest flocculating activity was selected and used for further studies.

2.6 Production of Bioflocculant Using Different Production Media

Production of bioflocculant by the selected isolates was investigated. Four different production media were used for bioflocculant production using six selected bioflocculant producing strains. The initial pH of the media was adjusted to 7.0 by NaOH (0.1M) and HCI (0.1M). The medium include: Medium 1 (M1) with the following composition : 10 g of glucose, 1.0 g of peptone, 0.3 g of $MgSO_4$ .7 H_2O, 5 g of K_2HPO_4 and 0.2 g of KH_2PO_4 in 1 liter of distilled water (Zhang *et al*., 2007 modified by Cosa *et al*., 2011); Medium 2 (M2) with the following composition: 20g glucose, 0.5g urea, 0.5g yeast extract, 0.2g $(NH_4)_2SO_4$, 5.0g K_2HPO_4, 2 g KH_2PO_4, 0.1, NaCl and 0.2g $MgSO_4$ .7 H_2O in 1litre of distilled water (Zhang *et al*., 2006); Medium 3 (M3) with the following composition: 20.0 g glucose, 2.0 g KH_2PO_4, 5.0 g K_2HPO_4, 0.2 g $(NH_4)_2SO_4$, 0.1g NaCl, 0.5 g urea and 0.5 g yeast extract per litre of deionized water (Wang

et al., 1995; Zheng *et al*., 2008) and Medium 4 (M4) with the following composition: 10g of glucose, 7.0g NH_4Cl, 0.5g K_2HPO_4, 0.5g $MgSO_4.7\ H_2O$, 40mg $FeCl_3$, 150mg $CaCl_2$ and 140mg $MnSO_4$ (Zaki *et al*., 2011). The medium was sterilized, inoculated with pure culture of the isolate and incubated on a rotary shaker at 120 rpm and 37^oC for 3 days. Kaolin suspensions at a concentration of 5,000mg/l were then used to evaluate the flocculating capability of a series of the culture broths.

2. Development of dynamic composting of processes simulation model

Most modern municipal solid waste composting operations emphasize the enhancement of decomposition rate of the organic matter as well as the economic operating cost. This can be achieved once the composting process kinetics is well understood. Based on microbial process kinetics, mass conservation equation, energy conservation equation and water balance, differential equations describing microbial, substrate, oxygen concentrations, moisture content and temperature profiles are derived. Then a simulation model for domestic solid waste composting processes is developed. The process is shown in Figure 1.

1. Results

In this study, a total of thirty nine (39) bacteria were isolated from three different wastewater samples (Brewery effluent collected from international Breweries Plc Omi-asoro, Ilesha, Palm-oil wastewater collected from Fiditi village in Afigio LGA, Ibadan, and Abattoir processing wastewater from Kara in Ibadan, western part of Nigeria, Osun and Oyo States respectively).

The isolates were screened for bioflocculant production using kaolin solution (5g/L) as a test material. The bacteria were screened for the production of bioflocculant as shown in Table 1. There was a significant different (P≥0.05) in the flocculating ability of the isolates. The percentage flocculating activity ranged from 03.00^p – 75.63^a % in which isolate TB3 had the highest followed in order by isolates TM3, AM6 and API, while isolate AP3 had the least.

The pH during fermentation ranged from 5.03^q -7.69^a in which isolate AM2 follow in order by AM11, AM1, AM10, AP2, NB5, TB2 and NB1 was able to reduce the initial pH of the medium from 7.0 to weakly acidic pH, while isolates NB1, TM6, AM12, TB3, TM1 and TM4 causes an increase in the pH of the medium.

Six isolates with the best flocculating activity after screening were selected and characterized. Table 2 shows the biochemical and physiological characterization of the selected isolates with their probable identities. The probable organisms were: *Enterobacter asburiae* AM6, *Streptococcus plurextorum* AP1, *Solibacillus silvestri* AP4, *Bacillus licheniformis* NB2, *Citrobacter* sp. TB3 and *Paenibacillus polymyxa* TM3 respectively.

Based on the flocculating activity after screening six (6) isolates with flocculating activity greater than 67% were selected for the production of bioflocculant using different media.

Four (4) different bioflocculant production media (BPM) were used to determine the effect of media compositions on the flocculating ability of the selected bacteria isolates at different incubation time. There was a significant difference (P≥0.05) in the flocculating ability of the isolates in different production medium.

There was a significant difference (P≥0.05) in the flocculating activity of the isolates in production Medium 1. The percentage flocculating activity ranged from 31.0 -89.19% in which isolate AP4 had the highest at 48hrs after incubation. At 48hrs of incubation about 33.5% of the isolate (AP4 and TB2) had flocculating activity above 70% while about 16.7% of the isolate (AM6) had flocculating activity above 70% at 72hrs of incubation as shown in Figure 1.

Figure 2 shows the percentage flocculating activity of the isolates in production Medium 2. There was a significant difference (P≥0.05) in the flocculating activity of the isolates in the medium. The percentage flocculating activity ranged from 32.91 -89.19% in which isolate AP4 had the highest at 48hrs after incubation. At 48hrs of incubation about 83.3% of the isolate (AM6, AP1, AP4, NB2 and TB2) had flocculating activity above 70% while about 16.7% of the isolate (AP4) had flocculating activity above 70% at 72hrs of incubation. At 48hrs isolate TM3 had the lowest flocculating activity.

There was a significant difference (P≥0.05) in the flocculating activity of the isolates in the Medium 3. The percentage flocculating activity of the isolates in production Medium 3 is shown in Figure 3. It ranged from 34.81 -78.38% in which isolate AP4 had the highest at 48hrs after incubation. At 48hrs of incubation about 66.7% of the isolate (AM6, AP1, AP4 and TB2) had flocculating activity above 70% while about 16.7% of the isolate (AP4) had flocculating activity above 70% at 72hrs of incubation.

There was a significant difference (P≥0.05) in the flocculating activity of the isolates in Medium 4. Figure 4 shows the percentage flocculating activity of the isolates in production Medium 4. It ranged from 34.65 -88.02% in which isolate NB2 had the highest at 48hrs after incubation. At 48hrs of incubation about 66.7% of the isolate (AM6, AP1, AP4 and NB2) had

flocculating activity above 70% while about 33.3% of the isolate (AP4 and NB2) had flocculating activity above 70% at 72hrs of incubation. At 48hrs isolate TM3 had the lowest flocculating activity.

Table 1: Screening for Bioflocculant producing isolates

S/N	NAMES	pH		Percentage (%) Flocculation
		Initial	Final	
1	AM1	7.0	5.06^{p}	57.48^{g}
2	AM2	7.0	5.03^{q}	53.64^{h}
3	AM3	7.0	7.13^{f}	31.52^{n}
4	AM4	7.0	6.67^{ijk}	50.60^{j}
5	AM5	7.0	7.00^{gh}	61.19^{f}
6	AM6	7.0	6.57^{k}	69.54^{b}
7	AM7	7.0	7.22^{de}	06.56^{o}
8	AM8	7.0	7.39^{bc}	51.39^{i}
9	AM9	7.0	7.10^{f}	39.47^{m}
10	AM10	7.0	5.12^{op}	52.72^{i}
11	AM11	7.0	5.04^{p}	54.04^{h}
12	AM12	7.0	7.45^{b}	53.64^{h}
13	AM13	7.0	6.61^{jk}	54.04^{h}
14	AM14	7.0	6.44^{l}	52.72^{i}
15	**AP1**	7.0	6.74^{i}	69.14^{b}
16	AP2	7.0	5.16^{op}	07.81^{o}
17	AP3	7.0	6.73^{i}	03.00^{p}
18	**AP4**	7.0	6.85^{i}	68.21^{c}
19	AP5	7.0	7.21^{de}	46.23^{k}
20	NB1	7.0	5.93^{n}	60.00^{f}
21	**NB2**	7.0	6.02^{m}	67.28^{d}
22	NB3	7.0	6.72^{i}	65.03^{e}
23	NB4	7.0	6.65^{jk}	42.91^{l}
24	NB5	7.0	5.88^{o}	35.50^{m}
25	NB6	7.0	6.45^{l}	53.91^{h}
26	TM1	7.0	7.42^{b}	51.79^{i}
27	TM2	7.0	7.31^{cd}	46.75^{k}
28	**TM3**	7.0	7.40^{b}	69.54^{b}
29	TM4	7.0	7.40^{b}	52.19^{i}
30	TM5	7.0	7.21^{de}	65.70^{e}
31	TM6	7.0	7.69^{a}	52.85^{i}
32	NM1	7.0	5.91^{r}	52.19^{i}
33	NM2	7.0	7.28^{d}	58.41^{g}
34	TB1	7.0	6.72^{i}	44.37^{l}
35	TB2	7.0	5.95^{mn}	60.93^{f}
36	TB3	7.0	7.48^{b}	75.63^{a}
37	TB4	7.0	7.03^{g}	38.68^{m}
38	TB5	7.0	7.42^{b}	52.45^{i}
39	NP1	7.0	6.90^{h}	46.36^{k}

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

Table 2: Biochemical and physiological characterization of the selected isolates

ISOLATE	Gram staining	Cellular morphology	Spore staining	MR test	VP test	Catalase test	Oxidase test	Indole test	Citrate test	Nitrate reduction	Urease test	Starch hydro.	Glucose	Fructose	Galactose	Sucrose	Lactose	Maltose	Arabinose	Xylose	Mannitol	PROBABLE IDENTITY
AM6	+	C	-	+	-	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	*Enterobacter asburiae*
AP1	+	C	-	+	-	+	+	-	+	-	-	-	+	-	+	-	-	-	-	+	-	*Streptococcus massiliensis*
AP4	+	R	-`	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	*Streptomyces sp.*
NB2	+	R	+	+	-	+	+	-	+	-	-	-	+	+	-	+	-	-	-	+	-	*Bacillus endophyticus*
TB3	-	R	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	+	+	*Citrobacter sp.*
TM3	+	R	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	*Lysinibacillus sphaericus*

Key: + = Positive; - = Negative, R = Rod shape; C = Cocci shape, MR = Methyl red; VP = Voges proscauer

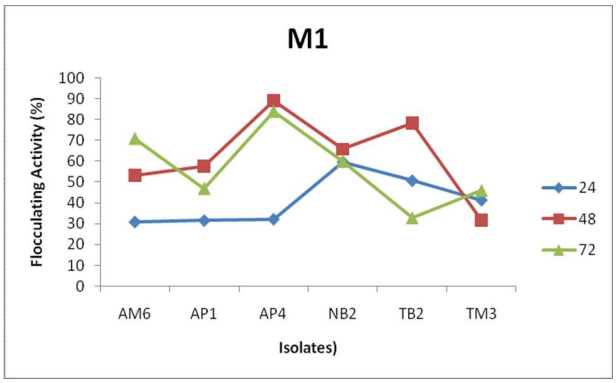

Figure 1 Flocculating activity of the selected bioflocculant producing isolates in Medium 1

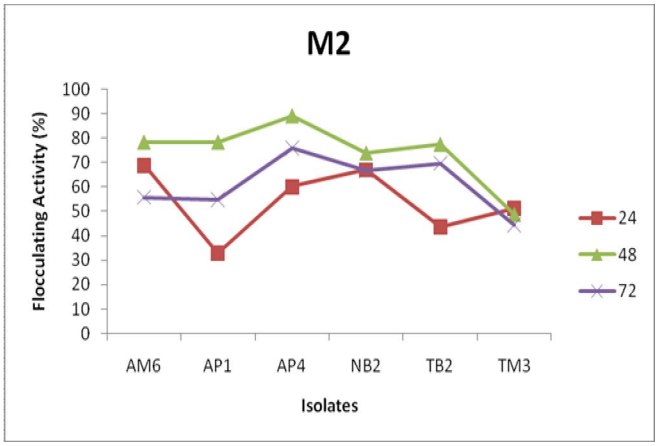

Figure 2 Flocculating activity of the selected bioflocculant producing isolates in Medium 2

Figure 3 Flocculating activity of the selected bioflocculant producing isolates in Medium 3

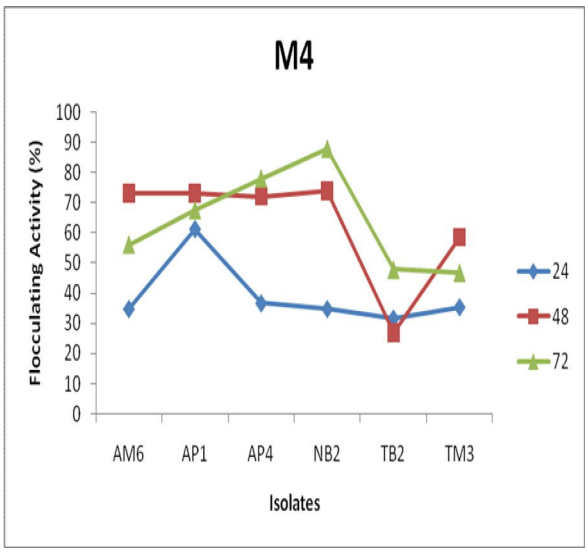

Figure 4 Flocculating activity of the selected bioflocculant producing isolates in Medium 4

4. Discussion

The uniqueness in the chemical composition of bioflocculant (i.e. protein, polysaccharide and glycoprotein production) and biodegradability of microbial flocculants have prompted research into screening, characterization and structural identification of polymeric flocculants elaborated by the microbes globally (Deng *et al*., 2003). In this research, out of the thirty nine (39) bacteria isolated from wastewater samples (Brewery, Palm oil and Abattoir wastewaters) in western part of Nigeria (Osun and Oyo States), only Six had high percentages of flocculating activities above sixty seven percentage (>67%).

The four (4) different production media used as basal medium for bioflocculant production had a profound effect on flocculating activity of the isolates. These effects may be as a result of the chemical composition of the basal media. It has been reported that constituents of the culture medium and culture conditions have a profound effect on bioflocculant production by some microorganisms (He *et al.,* 2004, Xia *et al*., 2008)

Glucose which was the major carbon source in the media supported the flocculating ability of the isolates. All the isolates grew and produced a reasonable flocculants when glucose was used as the sole carbon source. This result agreed with the report of Cosa *et al.,* (2011) who reported that using glucose as carbon source yielded bioflocculant with the highest flocculating activity (70.4%),

About 33.5% of the isolate (AP4 and TB2) had flocculating activity above 70% at 48hrs of incubation in Medium 1. This is an indication that Medium 1 is not a good production medium for most of the isolates. Inability of this medium to support flocculation ability of most of the isolates may be as a result of the fact that the medium contain only peptone as the only sole nitrogen sources. This result is not in agreement with the report of Cosa *et al*., (2011) who reported that peptone was preferred nitrogen source as it resulted in production of bioflocculant with the highest flocculating activity (70.4%) compared to other nitrogen sources. Gong *et al*., (2008) reported that peptone and other nitrogen sources were not favorable for bioflocculant production.

About 83.3% of the isolate (AM6, AP1, AP4, NB2 and TB2) had flocculating activity above 70% in production Medium 2. Ability of the isolates to produce higher flocculating activity in Medium 2 can be as a result of the presence of organic (Urea and yeast extract a) and inorganic ($(NH_4)_2SO_4$) nitrogen sources. This result is in contrast to the findings of Gong *et al*., (2008) who reported that peptone and other nitrogen sources were not favorable for bioflocculant production. The presence of monovalent and divalent ions in the basal media had a profound influence on the flocculating activity of the isolates. The presence of monovalent cation (NaCI) and divalent cation ($MgSO_4$) in Medium 2 could have accounted for the ability of the medium to support higher flocculating activity of the isolates after 48hrs of incubation.

About 66.7% of the isolate (AM6, AP1, AP4 and TB2) had flocculating activity above 70% in production Medium 3. Ability of Medium 3 to support flocculating activity of some isolates may be as a result of the presence of both organic and inorganic sources of nitrogen in the medium.

About 66.7% of the isolate (AM6, AP1, AP4 and NB2) had flocculating activity above 70% in production Medium 4. This may be as a result of the

fact that Medium 4 contain inorganic source of nitrogen ($(NH_4)_2SO_4$) which can easily be metabolized by the isolates during metabolism and synthesis of bioflocculant. The presence of divalent cations ($MgSO_4$, $FeCI_3$, $CaCI_2$ and $MnSO_4$) may contribute to the supportive ability of medium 4 on higher flocculating activity of about 66.7% of the isolates. This result is in agreement with the report of Sheng *et al.,* (2006) and Li *et al.,* (2008). Cosa *et al*., (2011) also reported that the presence of iron sulphate enhanced bioflocculant production by *Virgibacillus* sp. Rob. The requirement for the co-presence of both monovalent and divalent cations has been reported for enhancing flocculating efficiency by *Aeromonas* sp. (Li *et al.,* 2008).

In conclusion, the action of bacteria converts the simple substances in their environment into complex polymers that can be used as flocculant (Zheng *et al*., 2008). The results of the experiments demonstrated that the bioflocculants produced from the selected strains could widely be applied in different industrial processes, including wastewater treatment, downstream processing, food and fermentation processes. Despite the effective flocculation performance and low cost of the synthetic chemical flocculants, their use has resulted in some health and environmental problems. It is therefore recommended that flocculating agents produced by microorganisms should be put into use for the treatment of wastewaters.

Corresponding Author:
Adebayo-Tayo Bukola Christianah
Department of Microbiology, Faculty of Science,
University of Ibadan, Ibadan, Oyo state, Nigeria.
Email: bukola_tayo@gmail.com
Tel: 234 803 5522409

Reference

2. Cosa, S., Mabinya, LV., Olaniran, AO., Okoh, OO., Bernard, K., Deyzel, S. and Okoh, AI. 2011. Bioflocculant production by *Virgibacillus* sp. Rob isolated from the bottom sediment of Algoa Bay in the Eastern Cape, South Africa. *Molecules.* **16:** 2431-2442.
3. Deng, S. B., Bai, RB. Hu, XM. and Luo, Q., (2003).Characteristics of a bioflocculant produced by Bacillus mucilaginosus and its use in starch wastewater treatment. Appl. Microbiol. Biotech., 60 (5), 588–593.
4. Droste, RL. 1997. Theory and Practice of Water and Wastewater Treatment. John Wiley and Sons, New York, pp. 384-415.
5. Eguchi, MT. Nishikawa, K. Macdonald, R. Cavicchioli, JC. Gottschal and Kjelleberg, S. 1996. Responses to stress and nutrient availability by the marine *ltramicrobacterium Sphingomonas* sp. Strain RB2256. Appl. Environ. Microbiol., 62: 1287-1294.
6. Ferenci, T. 1999. Regulation by nutrient limitation. Curr. Microbiol., 2: 208-213.
7. Ferenci, T. 2001. Hungry bacteria – definition and properties of a nutritional state. Appl. Environ. Microbiol., 3: 605-611.
8. Gao, Q., Zhu, XH., Mu, J., Zhang, Y. and Dong, XW. 2009. Using Ruditapes philippinarum conglutination mud to produce bioflocculant and its applications in wastewater treatment. Bioresour. Technol; 100 4996-5001.
9. Gao, J., Bao, HY., Xin, MX., Liu, Y.X.., Li, Q. and Zhang, YF. 2006. Characterization of a bioflocculant from a newly isolated Vagococcus sp. W31. J. Zhejiang Univ. Sci. B 7, 186–192.
10. Grady, CPL., Smets BF. and Barbeau. DS. 1996. Variability in kinetic parameter estimates: A review of possible causes and a proposed terminology. Wat. Res., 3: 742-748.
11. Gong, W., Wang, S., Sun, F., Liu, XW., Yue, QY., Gao, BY. 2008. Bioflocculant production by culture of *Serratia ficaria* and its application in wastewater treatment. Bioresour. Technol; 99, 4668–4674.
12. He, N., Li, Y., Chen, J. and Lun, SY. 2002. Identification of a novel bioflocculant from a newly isolated *Corynebacterium glutamicum*. Biochem. Eng. *J.* **11:** 137-148.
13. He, N., Li, Y., Chen, J. 2004. Production of a novel polygalacturonic acid bioflocculant REA-11 by *Corynebacterium glutamicum*. Bioresour. Technol.; 94, 99-105.
14. Kurane, R. and Nohata, Y. 1994. A new water-absorbing polysaccharide from *Alcaligene slatus*. Biosci. Biotechnol. Biochem.; 58, 236.
15. Kurane, R., Takeda, K., Suzuki, T. 1986. Screening and characteristics of microbial flocculants. Agric. Biol. Chem., 50, 2301–2307.
16. Lin, J. and Harichund, C.2011. Industrial effluent treatments using heavy-metal removal bacterial bioflocculants. *Short communication*. **37:** 2.
17. Li, X.M., Haung, QY., Zeng, G.M., Liao, X., Liu, JJ., Long, WF. (2007). Screening and characterization of a bioflocculant produced by *Aeromonas* sp. Biomed. Environ. Sci., 20,274-278.
18. Li, WW, Zhou, WZ., Zhang, YZ., Wang, J., Zhu, XB. (2008). Flocculation behavior and mechanism of an exopolysaccharide from the deep-sea psychrophilic bacterium *Pseudoalteromonas* sp. SM9913. Bioresour. Technol., 99, 6893-6899.
19. Leonard V. Mabinya, Sekelwa Cosa, Uchechukwu Nwodo and Okoh , A.I. 2012. Int.

J. Mol. Sci., 13, 1054-1065; doi:10.3390/ijms13011054
20. Nakata, K. and. Kurane, R. 1999. Production of extracellular polysaccharide bioflocccculants by *Klebsiella pneumoniae*. Journal of Bioscience, Biotechnology and Biochemistry. **63:** 2064-2068.
21. Sundstrom, DW. and Klei, HE. 1979. Wastewater Treatment. Prentice Hall, Englewood Cliffs, NJ, pp. 235-255.
22. Sheng, Y., Zhang, Q., Sheng, Y., Li, C., Wang H. 2006. Screening and flocculating properties of bioflocculant-producing microorganisms. Sci. Technol., 13, 289-292.
23. Taniguchi, M., Kato, K., Shimauchi, A., Ping, X., Fujita, K.I., Tanaka, T., Tarui, Y. and Hirasawa, E. 2005. Phisicochemical properties of cross-linked poly-γ-glutamic acid and its flocculating activity against kaolin suspension. J. Biosci. Bioeng. **99:** 130-135.
24. Vijayalakshmi, SP. and Raichur, AM. 2003. The utility of Bacillus subtilis as a bioflocculant for fine coal. Colloids Surfaces B 29 265-275.
25. Wang, Z., Wang, KX., Xie, YM., Yao, YL., 1995. Bioflocculant-producing microorganisms. Acta Microbiol. Sin., 35:121-129 (in Chinese).
26. Wang, Z.,Wang., Xie,Y. 1995. Bioflocculant Producing Microorganisms.*Acta Microbiol.Sin,* 35(2): 121-129.
27. Xia, S., Zhang, Z., Wang, X., Yang, A., Chen, L., Zhao, J., Leonard, D., Jaffrezic-Renault, N. 2008. Production and characterization of bioflocculant by *Proteus mirabilis* TJ-1. Bioresour. Technol. ; 99, 6520-6527.
28. Zaki, S., Farag, S., Abu, GE., Elkady, M., Nosier, M. and Abd El Haleem. 2011. Characterisation of bioflocculants produced by bacteria isolated from crude petroleum oil. *Int. J. Environ. Sci. Tech,* **8(4):** 831-840.
29. Zhang, ZQ., Lin, B., Xia, SQ., Wang, X.J. and Yang, A.M. 2007. Production and application of a novel bioflocculant by multiple microorganism consortia using brewery wastewater as carbon source. *J. Environ. Sci*. **19:** 667-673.
30. Zheng, Y., Ye, ZL, Fang, XL., Li, YH. and Cai, WM. 2008. Production and characteristics of a bioflocculant produced by *Bacillus* sp. F19. Bioresour.. Technol. **99:** 7686-7691.

2/5/2013