Journal of Science Research

Screening and the effects of production conditions on bioflocculants' production by microorganisms isolated from wastewater

Ajani, T. F., Adebayo-Tayo, B. C.* and **Alao, S. O.**
Department of Microbiology, University of Ibadan, Ibadan
*Corresponding author: bukola.tayo@gmail.com

Abstract

Bioflocculants are biopolymers produced by microorganisms and used for removal of colloidal particles from solution. Screening, production and the effect of physicochemical conditions on bioflocculants production by microorganisms isolated from wastewater samples were investigated. Fifty-five (55) bacterial strains isolated from four different wastewater samples were screened for bioflocculants production. The flocculating activity ranged from 3.29-74.15% in which *Streptomyces* sp. HDW7 gave the highest. The best four bioflocculant producers, namely: *Streptomyces* sp. HDW7 (74.15%), *Nocardia* sp. OX5 (69.02%), *Bacillus licheniformis* PO7 (61.00%) and *Micrococcus* sp. PO3 (57.04%) were selected for further studies.30ºC, pH 7.0, and 72 hours supported the highest bioflocculants production. Bioflocculants production under different carbon and nitrogen sources ranged from 79.00-88.00% and 61.00-87.00% in which maltose and peptone supported the highest by 75% of the isolates (3 of the 4). Glucose and peptone supported the highest bioflocculant production (87.00%) by *Streptomyces* sp. HDW7. Bioflocculants production under static and agitation condition ranged from 36.66-58.33% and 60.66-87.33% with *Streptomyces* sp. HDW7 having the highest bioflocculant activity. Bioflocculants production using single and the consortium strains ranged from 72.33 – 81.67% in which single strain, *Streptomyces* sp. HDW7 had the highest followed by consortium of *Streptomyces* sp. HDW7 and *Nocardia* sp. OX5. In conclusion, some microorganisms isolated from the wastewater had a high potential for bioflocculants production. 30ºC, pH 7.0, 72 hours, glucose and peptone supported the highest bioflocculants production by the selected bioflocculant producing isolates.

Keywords: *Streptomyces* sp. HDW7; *Bacillus licheniformis* PO7; bioflocculants; flocculating activity; consortium.

Introduction

Water, despite being a valued and life essential natural resource, is relatively scarce and unavailable in some parts of the world. The quality of available water resources is also declining globally as a result of industrial and urban conurbations [1, 2].

Wastewater is any water that has been adversely affected in quality with a wide range of potentially harmful contaminants and pollutants in a concentration that the water is unfit for any usage [3]. Security of water supply then becomes a key strategic issue for continuing and sustaining economic growth and development.

Flocculation is a process by which suspended solid particles accumulate and coagulate into larger aggregates of flocs so that they can be easily separated from wastewater [4]. There are three types of flocculating agents viz: inorganic flocculants such as aluminium sulphate (popularly called alum), synthetic organic flocculants such as polyacrylamide derivatives and natural flocculants otherwise called bioflocculants [5]. Bioflocculants are biodegradable macromolecules secreted by many microorganisms (eubacteria, actinomycetes, fungi and even algae) during their growth [6]. They are innocuous, environmentally friendly, nontoxic and biodegradable with no secondary pollution from degradation intermediates and as such they can serve as better potential alternatives to existing

chemical flocculants [7, 8]. There are several reports on the potential of various species of microorganisms that are able to absorb suspended particles and metals from wastewater [9, 10]. Although majority of microbial bioflocculants that have been extracted come from *Bacillus* species [5, 11-13], He *et al* [14] investigated the production of a polygalacturonic acid bioflocculant REA-11 from isolated *Corynebacterium glutamicum* CCTCC M201005 strain, while Mona [15] investigated the production of bioflocculant by the marine actinomycete *Nocardiopsis aegyptia*.

But, the microbial biotechnological approaches for the production of high yield bioflocculants with effective flocculating abilities lies in the possibility of using different microorganisms to synthesize extracellular substances with different composition [13,16]. Comparative effect of medium composition, fermentation conditions and nutritive compounds of cultivation medium has been reported to have an influence on bioflocculant production by microorganisms [14,17].

Due to low flocculating capability and the high cost of production, industrial production of bioflocculants has not been established. Therefore, there is a need to search for microorganisms with a great capacity for bioflocculants production and reduction in cost of production [18], hence this research aimed at screening for bioflocculants producing strains from waste water samples and to study the effects of production conditions on bioflocculants production.

Materials and methods

Collection of samples

Wastewater samples were randomly collected from different sources viz: domestic wastewater and oxidation pond both in the University of Ibadan; palm-oil effluent in Ifon Osun, Osun State and abattoir effluent. The samples were collected using sterile containers and immediately transported to the laboratory for further analysis.

Isolation of microorganisms associated with the wastewater samples

Isolation of microorganisms associated with the effluents was carried out using serial dilution technique according to Harrigan and McCance [19]. 1 mL of the diluents poured and plated on Tryptone Soy Agar (TSA) for the isolation of *Bacillus* strains and Actinomycetes Isolation Agar (AIA) and Nutrient Agar (NA) were used for the isolation of actinomycetes and heterotrophs respectively. The inoculated plates were incubated and distinct colonies on the plates were subcultured to obtain a pure culture. The pure isolates obtained were preserved in agar slant and kept refrigerated for further use.

Screening of the isolates for bioflocculant producing potential

Fifty-five (55) strains isolated from the samples were screened for bioflocculant-producing potential using a Bioflocculant Production Medium with the composition: 10 g glucose, 2 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.5 g $CaCO_3$, and 0.5 g yeast extract dissolved in 1Litre deionized water with initial pH adjusted to 7.0 [13, 20].

Standardized seed culture (0.5 mL) of each isolate was inoculated into sterile 50mL (1% v/v) of the bioflocculant production broth (BPB) and incubated on a rotary shaker 160 rpm at 28°C for 72 hours. After incubation, flocculating activity was determined spectrophotometrically using cell-free culture supernatant and 5 g/L kaolin suspension [13]. The best four strains with the highest flocculating activity were selected for further study.

Determination of flocculating activity

This was carried out according to the method of Gao *et al* [18] using kaolin clay suspension as test material. The 5g/L kaolin clay at pH 7 was 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/control tube in which the culture supernatant replaced with distilled water was also included and measured under the same conditions. The final volume of all mixtures made up to 10 mL with distilled water. After vortexed for 1 minute, the solutions were allowed to settle for 5 minutes at room temperature. The optical density (OD) of the clarifying upper phase solution was measured at 550 nm with a UV-V is spectrophotometer and the flocculating activity determined as follows:

$$\text{Flocculating rate (\%)} = \{(B - A) / B\} \times 100 \text{ \%} \quad [21].$$

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

Effects of physicochemical parameters on bioflocculants production by the selected isolates

The effects of: pH (5.0-9.0), by appropriately adjusting with either 1.0 N HCl or NaOH solutions [22], temperature (20°C, 30°C, 35°C, 40°C, and 45°C),

incubation time (24 hours, 48 hours, 72 hours and 96 hours), carbon sources (glucose, sucrose, ethanol, maltose, and starch), nitrogen sources (peptone, yeast extract, urea, ammonium sulphate and potassium nitrate), static and agitation condition as well as single and consortium inoculation on bioflocculants production by the selected isolates were investigated.

Extraction and purification of the bioflocculants produced

The extraction of each bioflocculant was carried out according to the method of Nakata and Kurane [23]. The fermentation broth was centrifuged at 4,000 rpm for 30 minutes at 4°C to remove cells. One volume of distilled water was added to the supernatant and centrifuged again for 15 minutes to remove insoluble solutes. Two volume of cold ethanol was added to the supernatant and the solution was kept in the refrigerator at 4°C for 12 hours. The resultant precipitate was vacuum dried to obtain the crude bioflocculant. The crude product was weighed and dissolved in a small volume of distilled water and one volume of 2% Cetylpyridinium chloride (CPC) solution was added. After mixing, the mixture was kept at room temperature for 12 hours. The upper phase was centrifuged at 4,000 xg for 15 minutes and the supernatant was dialyzed against distilled water. Thereafter, the dialysate was vacuum dried to obtain a pure bioflocculant.

Statistical analysis

All experiments were performed in triplicate and the results subjected to one-way analysis of variance (ANOVA) using Minitab Student Release 12 Statistical Package (Minitab Inc., State College, PA, USA). Statistically, significant means were separated using Duncan multiple range test (DMRT) and Fisher pairwise comparisons (FPC). A significance level of $p \leq 0.05$ was used to reject the null hypothesis.

Results and discussion

Screening for bioflocculants producing isolates

Screening for bioflocculants-producing isolates is shown in Table 1. A total of fifty-five (55) isolates (bacteria and actinomycetes) obtained from four different wastewater samples were screened for the production of bioflocculants.

There was a significant difference ($p \leq 0.05$) in the flocculating ability of the isolates. The percentage flocculating activity ranged from 03.29±0.05-74.15±0.07%. The highest flocculating activity was recorded by *Streptomyces* sp. HDW7 isolated from domestic wastewater followed in order by *Nocardia* sp. OX5, *Bacillus licheniformis* PO7 and *Micrococcus* sp. PO3. These best four (4) isolates with the highest flocculating activity were selected for further studies.

Morphological and biochemical characterization of the selected bioflocculants producing isolates

The morphological and biochemical characterization carried out on the best four selected bioflocculant producers with their probable identities are shown in Table 2. Isolates HDW7, OX5, PO3 and PO7 have probable identities as *Streptomyces* sp., *Nocardia* sp., *Micrococcus* sp. and *Bacillus licheniformis* respectively.

Effect of pH on bioflocculants production by the selected isolates

The effect of pH on bioflocculants production by the selected isolates is shown in Figure 1. There was a significant difference ($p \leq 0.05$) in the flocculating activity of the isolates at different pH. The flocculating activity of *Streptomyces* sp. HDW7 ranged from 78^{d}-87^{d}% in which the highest was recorded at pH 7.0 and the least at pH 5.0. For *Nocardia* sp. OX5, it ranged from 73^{c}-79^{c}% with the highest recorded at pH 7.0 and the least at both pH 5.0 and 9.0. For *Micrococcus* sp. PO3, it ranged from 59^{a}-64^{b}% with the highest recorded at pH 5.0 and the least at pH 9.0. For *Bacillus licheniformis* PO7, it ranged from 52^{a}-72^{b}% with the highest recorded at pH 7.0 and the least at pH 6.0.

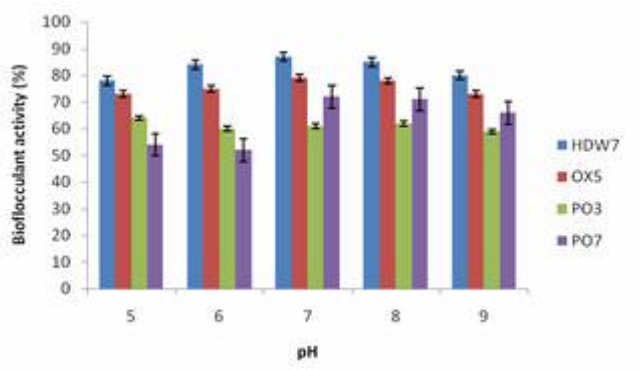

Figure 1. Effect of pH on bioflocculants' production by the selected isolates.

Key: HDW7: *Streptomyces* sp. HDW7, OX5: *Nocardia* sp. OX5, PO3: *Micrococcus* sp. PO3, PO7: *Bacillus licheniformis* PO7.

Table 1. Screening for bioflocculants-producing isolates.

S/N	Isolate	% Flocculation	S/N	Isolate	% Flocculation
1.	HDW2	42.94±0.03	36	PO7a	28.03±0.01
2.	HDW4	26.95±0.01	37	PO7b	41.00±0.04
3.	BAC5	09.00±0.00	38	PO7c	31.99±0.25
4.	BAC6	47.53±0.01	39	BAC1	19.99±0.23
5.	BAC7	47.53±0.02	40	BAC2	25.02±0.00
6.	OX6b	54.26±0.05	41	BAC3	45.98±0.03
7.	OX7a	38.48±0.02	42	BAC4	36.01±0.05
8.	OX7b	11.02±0.01	43	FRN5	15.00±0.01
9.	OX7c	25.17±0.06	44	PO6c	19.02±0.01
10.	ABW2	44.00±0.02	45	DS5c	16.46±0.00
11.	ABW3	06.58±0.03	46	DS7c	O4.72±0.03
12.	FRN7	07.41±0.00	47	DS8c	29.08±0.01
13.	HDW8	30.98±0.07	48	PO3	57.04±0.04
14.	HDW5a	06.53±0.00	49	PO5	24.98±0.04
15.	HDW5b	39.25±0.03	50	PO6a	35.00±0.03
16.	NH3	16.32±0.00	51	PO6b	24.01±0.04
17.	NH4	27.00±0.04	52	PO8a	45.97±0.02
18.	HDW7	74.15±0.07	53	PO8b	39.01±0.00
19.	HDW9	33.20±0.02	54	PO7	61.00±0.06
20.	NH5a	20.18±0.01	55	ABW7	03.29±0.05
21.	NH5b	13.99±0.13			
22.	NH6	20.38±0.11			
23.	NH7	37.22±0.04			
24.	OX5	69.02±0.09			
25.	OX6	14.71±0.00			
26.	OX7	36.98±0.03			
27.	PO8	16.05±0.04			
28.	DS6b	22.02±0.00			
29.	DS8	40.03±0.02			
30.	PO9	10.97±0.01			
31.	PO6d	16.02±0.02			
32.	ABW6	39.98±0.05			
33.	FRN6	41.00±0.03			
34.	FW7a	16.05±0.12			
35.	FW7b	38.00±0.09			

Values are mean with standard error significantly different at $p \leq 0.05$ using Duncan Multiple Range Test (DMRT) and Fisher Pairwise Comparisons (FPC) for separation of statistically significant means.

Effect of temperature on bioflocculants production by the selected isolates

The effect of incubation temperature on bioflocculants-production by the selected isolates is shown in Figure 2. There was a significant difference ($p \leq 0.05$) in the flocculating activity by the isolates at different incubation temperature. The flocculating activities of *Streptomyces* sp. HDW7, *Nocardia* sp. OX5 and *Bacillus licheniformis* PO7 ranged from 53^{a}-87^{e}%; 49^{a}-81^{d} % and 46^{a}-70^{c}% respectively in which the highest were recorded at 30^{o}C and the least at 45^{o}C. For *Micrococcus* sp. PO3, it ranged from 44^{a}-65^{a}% with the highest recorded at 35^{o}C and the least at 45^{o}C.

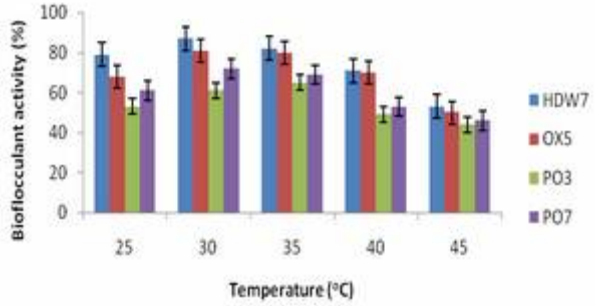

Figure 2. Effect of temperature on bioflocculants-production by the selected isolates.

Key: HDW7: *Streptomyces* sp. HDW7, OX5: *Nocardia* sp. OX5, PO3: *Micrococcus* sp. PO3, PO7: *Bacillus licheniformis* PO7.

Table 2. Morphological and biochemical characterization of the selected bioflocculant producing isolates.

Biochemical test	HDW7	OX5	PO3	PO7
Growth degree	Abundant	Moderate	Moderate	Abundant
Colony form	Concave, Wooly	Flat	Round	Irregular
Aerial mycelium	Greenish-Yellow	White	ND	ND
Substratemycelium	Yellow	Grayish green	ND	ND
Diffusible pigment	Brown	Nil	Nil	Nil
Shape	C	R	C	R
Gram stain	+	+	+	+
Spore stain	+	+	-	+
Catalase	+	+	+	+
Oxidase	+	+	-	+
MR	+	-	-	+
VP	-	+	-	-
Indole	-	+	-	-
Citrate	+	-	+	+
Nitrate reduction	+	-	-	-
Urease	+	+	-	-
Starch hydrolysis	+	-	-	-
Glucose	+	+	-	+
Sucrose	-	-	-	+
Lactose	-	-	-	-
Maltose	+	-	-	-
Mannitol	+	+	-	-
6.5% NaCl	ND	ND	ND	+
Growth at 55^{o}C	ND	ND	ND	+
Probable identity	*Streptomyces* sp.	*Nocardia* sp.	*Micrococcus* sp.	*B. Licheniformis*
Isolate code	HDW7	OX5	PO3	PO7

Key: + = Positive; – = Negative; C = Cocci; R = Rod; MR = Methyl red; VP = Vogesproscauer; ND: Not detected.

Effect of fermentation time on bioflocculants production by the selected isolates

The effect of different fermentation time on bioflocculants-production by the selected isolates is shown in Figure 3. There was a significant difference ($p \leq 0.05$) in the flocculating activity by the isolates at different fermentation time. The flocculating activity of *Streptomyces* sp. HDW7 ranged from 46^{d}-87^{d}% in which the highest was recorded at 72 hours and the least at 24 hours. For *Nocardia* sp. OX5, it ranged from 37^{b}-79^{c}% with the highest recorded at 72 hours and the least at 96 hours. For *Micrococcus* sp. PO3, it ranged from 36^{a}-61^{a}% with the highest recorded at 72 hours and the least at 24 hours. For *Bacillus licheniformis* PO7, it ranged from 29^{a}-72^{b}% with the highest recorded at 72 hours and the least at 96 hours.

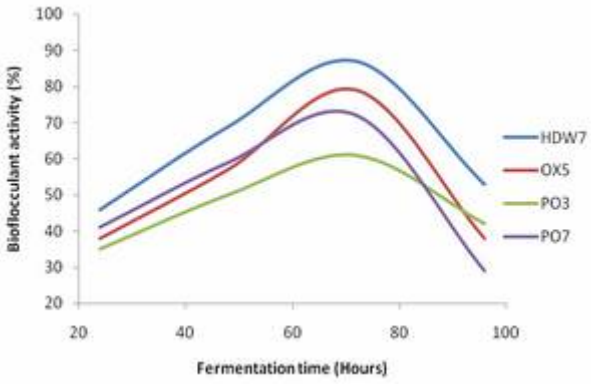

Figure 3. Effect of fermentation time on bioflocculants' production by the selected isolates.

Key: HDW7: *Streptomyces* sp. HDW7, OX5: *Nocardia* sp. OX5, PO3: *Micrococcus* sp. PO3, PO7: *Bacillus licheniformis* PO7.

Effect of carbon sources on bioflocculants production by the selected isolates

The effect of different carbon sources on bioflocculants-production by the selected isolates is shown in Figure 4. There was a significant difference (pd"0.05) in the flocculating activity by the selected isolates in different carbon sources. The flocculating activity of *Streptomyces* sp. HDW7 ranged from 50^{d}-87^{a}% in which the highest was recorded in glucose and the least in ethanol. For *Nocardia* sp. OX5 it ranged from 40^{b}-88^{a}% with the highest recorded in maltose and the least in sucrose. For *Micrococcus* sp. PO3, it ranged from 38^{b}-78^{c}% with the highest recorded in maltose and the least in sucrose. For *Bacillus licheniformis* PO7, it ranged from 39^{a}-81^{c}% with the highest recorded in maltose and the least in starch.

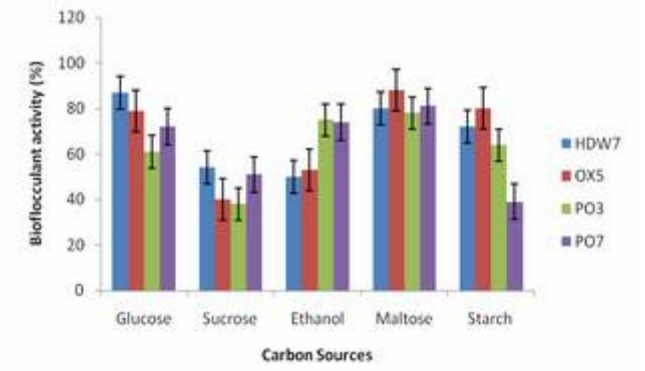

Figure 4. Effect of carbon sources on bioflocculants-production by the selected isolates.

Key: HDW7: *Streptomyces* sp. HDW7, OX5: *Nocardia* sp. OX5, PO3: *Micrococcus* sp. PO3, PO7: *Bacillus licheniformis* PO7.

Effect of nitrogen sources on bioflocculants production by the selected isolates

The effect of different nitrogen sources on bioflocculants-production by the selected isolates is shown in Figure 5. There was a significant difference (pd"0.05) in the flocculating activity by the isolates in different nitrogen sources. The flocculating activities of *Streptomyces* sp. HDW7, *Micrococcus* sp. PO3 and *Bacillus licheniformis* PO7 ranged from 51^{b}-87^{d}%, 39^{a}-61^{a}% and 43^{a}-72^{b}% respectively in which the highest were recorded in peptone and the least in potassium nitrate. For *Nocardia* sp. OX5, it ranged from 66^{c}-82^{d}% with the highest recorded in urea and the least in potassium nitrate.

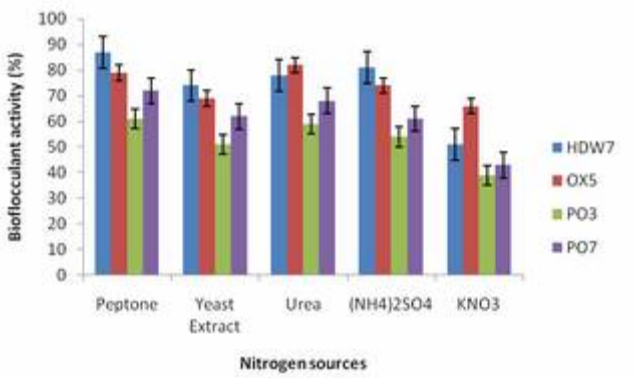

Figure 5. Effect of nitrogen sources on bioflocculants' production by the selected isolates.

Key: HDW7: *Streptomyces* sp. HDW7, OX5: *Nocardia* sp. OX5, PO3: *Micrococcus* sp. PO3, PO7: *Bacillus licheniformis* PO7.

Effect of static and agitation on bioflocculants production by the selected isolates

The effect of static and agitation conditions on bioflocculants-production by the selected isolates is shown in Figure 6. There was a significant difference (pd"0.05) in the flocculating activity of the selected isolates. In static incubation, the flocculating activity ranged from 37^{a}-58^{d}% with the highest recorded by *Streptomyces* sp. HDW7 and the least by *Bacillus licheniformis* PO7.

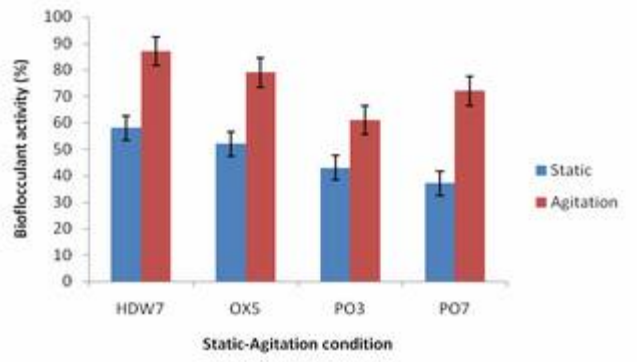

Figure 6. Effect of static and agitation condition on bioflocculants' production by the selected isolates.

Key: HDW7: *Streptomyces* sp. HDW7, OX5: *Nocardia* sp. OX5, PO3: *Micrococcus* sp. PO3, PO7: *Bacillus licheniformis* PO7.

During agitation, the flocculating activity ranged from 61^{a}-87^{d}% with the highest recorded by *Streptomyces* sp. HDW7 and the least by *Micrococcus* sp. PO3.

Effect of single and consortium inoculation on bioflocculants production by the selected isolates

The effect of single strain and consortium inoculation

on bioflocculants-production by the selected isolates is depicted in Figure 7. There was a significant difference ($p \leq 0.05$) in the flocculating activity when the selected axenic strains were used as a single starter and in the consortium. The flocculating activity ranged from 72^{a}-82^{c}% in which the highest was recorded by single strain *Streptomyces* sp. HDW7, followed by the consortium of *Streptomyces* sp. HDW7 and *Nocardia* sp. OX5; and the least by single strain *Nocardia* sp. OX5.

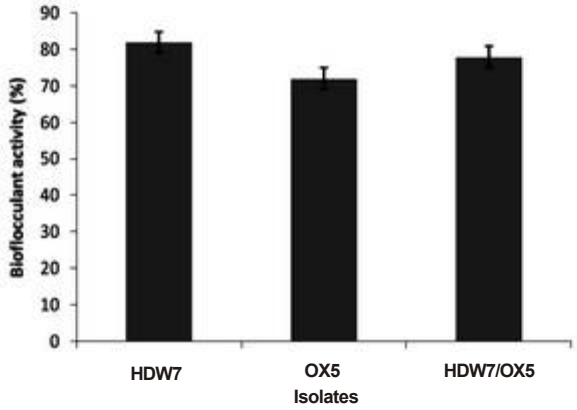

Figure 7. Effect of single and consortium strains on bioflocculants' production by the selected isolates.

Key: HDW7: *Streptomyces* sp. HDW7, OX5: *Nocardia* sp. OX5, HDW7/OX5: Consortium of *Streptomyces* sp. HDW7 and *Nocardia* sp. OX5.

As noted in previous studies, the pH for bioflocculants' accumulation varied with different microorganisms [24]. Bioflocculants' productions by the selected isolates were affected by the initial pH of the production medium. Neutral pH is the best for bioflocculants' production by *Streptomyces* sp. HDW7, *Nocardia* sp. OX5 and *Bacillus licheniformis* PO7. This result is in accordance with the works of Nwodo *et al* [25] on bioflocculants' production by *Streptomyces* sp. Gansen; Nwodo *et al*. [26] on bioflocculants' production by *Brachybacterium* sp; Adebami and Adebayo-Tayo [17] on bioflocculant production by *Streptomyces* sp. AP4 and Mona [14] on bioflocculant produced by *Nocardiopsis aegyptia* sp. nov but disagree with the works of Cosa *et al* [27] and Zhang *et al* [28] who respectively reported that *Virgibacillus* sp. Rob and *Streptomyces* sp. xn17 produced best bioflocculants under alkaline (pH 12.0) and acidic (pH 4.0) conditions. On the other hand, pH 5.0 supported the highest bioflocculant activity by *Micrococcus* sp. PO3. This result is similar to the work of Nwodo *et al* [25] who reported weak acidic condition as best supporting bioflocculant production by *Streptomyces* sp. Gansen.

The initial pH of the production medium determines the electric charge of the cells and the oxidation-reduction potential, which can affect adsorption of nutrients and enzymatic reaction for bioflocculants production [29,30].

Bioflocculants produced by *Streptomyces* sp. HDW7, *Nocardia* sp. OX5, *Micrococcus* sp. PO3 and *Bacillus licheniformis* PO7, were affected by an increase in the fermentation temperature. Generally, a sharp decrease in bioflocculants' production was observed after fermentation temperature of 35°C. Fermentation temperature of 30°C gave the highest bioflocculants' production by *Streptomyces* sp. HDW7, *Nocardia* sp. OX5 and *Bacillus licheniformis* PO7, while 35°C supported the highest bioflocculants production by *Micrococcus* sp. PO3. This result is similar to the work of Okoh *et al* [31] on bioflocculant production by *Methylobacterium* sp. isolated from marine environment, and the report of Adebami and Adebayo-Tayo [17] on the bioflocculant production by *Streptomyces* sp. AP4.

In the course of fermentation period, the correlation between bioflocculants' production and fermentation time differ among different micro-organisms. In this study, extension in fermentation period from 24 hours to 72 hours generally increases the bioflocculant activity until the maximum flocculating efficiency attained in the late exponential phase. This is possibly an indication that the bioflocculants were produced by biosynthesis during growth and not by cell autolysis [24]. Further increase in fermentation period resulted in a sharp decrease in bioflocculant activity and this could be attributed to accumulation of toxic metabolic wastes, cell autolysis and/or the presence of bioflocculant degrading enzymes [7]. This result is similar to that of bioflocculants produced by: *Bacillus* sp. AEMREG7 as reported by Okaiyeto *et al* [5], freshwater *Streptomyces* sp. Gansen reported by Nwodo *et al* [25], and *Streptomyces* sp. xn17 reported by Zhang *et al* [28]; in which bioflocculant production were all synchronous with cell growth and reached the maximum activity in the late exponential/early stationary phase of cell but contrary to the report of Shimofuruya *et al* [32] on bioflocculant produced by *Streptomyces griseus* and that of the work of Liu *et al* [33] who observed that bioflocculants' production was in the death phase of the cell. It is, therefore evident that the bioflocculant biosynthesis occurred

during different phases of the microbial growth for different micro-organisms [34].

Streptomyces sp. HDW7 best utilized glucose as carbon source while maltose is the most preferred carbon source by *Nocardia* sp. OX5, *Micrococcus* sp. PO3 and *Bacillus licheniformis* PO7 for bioflocculants' production. This result is in agreement with the reports of Patil *et al* [35] on bioflocculant produced by *Bacillus subtilis*, Cosa *et al* [27] on *Virgibacillus* sp. Rob, Mabinya *et al* [36] on bioflocculants' production by *Halomonas* sp. Okoh, as well as the report of Adebami and Adebayo-Tayo [17] on bioflocculants' production by *Streptomyces* sp. AP4. Patil *et al.* [35] reported that the bioflocculant produced by *Bacillus subtilis* is enhanced by glucose and disaccharide sucrose as carbon sources. But the result of this study disagree with the work of Gong *et al* [37] on bioflocculants' production by culture of *Serratia ficaria* and that of the report of Rawhia *et al* [38] on bioflocculants produced by bacterial isolates from Egyptian soil best supported by starch as the carbon source.

It has been well documented that nitrogen sources are important nutrient factors that enhance bioflocculant production [24]. In this study, peptone provided the best nitrogen source for bioflocculant production by *Streptomyces* sp. HDW7, *Micrococcus* sp. PO3 and *Bacillus licheniformis* PO7. Urea is the best nitrogen source for bioflocculant production by *Nocardia* sp. OX5. This is similar to the results obtained for bioflocculants' production by *Virgibacillus* sp. Rob [27] and *Proteus mirabilis* TJ-1 [39] but disagrees with the report of Rawhia *et al* [38] on bioflocculants produced by bacterial isolates from Egyptian soil with yeast extract as best nitrogen source.

For agitation and static conditions, maximum bioflocculant activity was recorded during agitation condition while static condition greatly reduced bioflocculants' production in all the four selected isolates. This result is similar to the work of Adebayo-Tayo and Adebami [20] on bioflocculant produced by *Bacillus clausii* NB2 but in contrast with the report of Salehizadeh and Shojaosadati [29] that sometimes due to agitation of the culture medium, bioflocculant yield by bacterial cells might be greatly reduced and it is, therefore imperative to investigate if there is need to agitate the culture medium or not. Increase in bioflocculants' activity by the selected isolates under agitation condition might be due to the fact that agitation ensures the circulation/ distribution of dissolved oxygen which thus affects nutrients absorption and enzymatic reaction [29].

The effect of single strain and consortium in this study showed that single strain *Streptomyces* sp. HDW7 had higher bioflocculants' activity than the consortium of *Streptomyces* sp. HDW7 and *Nocardia* sp. OX5 and this may probably due to inhibitory effect or competition for available nutrients by the combined strains. Bioflocculants are biodegradable macro-molecules secreted by microorganisms. They are exopolysaccharide metabolites extracted and purified from microorganisms by the biotechnological process [40].

Conclusion

Microorganisms isolated from wastewater samples are potential bioflocculant producers. 30°C, pH 7.0, 72 hours, glucose and peptone supported the highest bioflocculants' production by the selected strains. Appropriate use of these physicochemical conditions with suitable bioflocculant producers can enhance relatively inexpensive production of bioflocculants from a variety of microorganisms and thus microbial flocculants become a promising alternative to the more expensive, toxic and environmentally hazardous chemical flocculants.

References

[1] Coetzee, H., Winde, F. and Wade, P. W. 2006. An assessment of sources, pathways, meschanisms and risks of current and potential future pollution of water and sediments in gold mining areas of the Wonderfonteinspruit Catchment.*WRC Report No.1214/1/06.* Pretoria: Water Research Commission.

[2] WHO-UNICEFJMP (World Health Organization and UNICEF Joint Monitoring Programme). 2015. Report on Drinking Water and Sanitation update and MDG Assessment. *Joint Press Release* www.wssinfo.org.

[3] Turton, A. R. and Ashton, P. J. 2008. Basin closure and issues of scale: The southern African hydro politicalcComplex. *International Journal of Water Resources Development 24(2):* 305-318.

[4] Nwodo, U. U., Green, E., Mabinya, L. V., Okaiyeto, K., Rumbold, K., Obi, C. L. and Okoh, A. I. 2014. Bioflocculant production by a consortium of *Streptomyces* and *Cellulomonas* species and media optimization via surface response model. *Colloids Surface Biomolecules Biointerfaces, 116:* 257-264.

[5] Okaiyeto, K., Uchechukwu, U. N., Mabinya, L. V., Arinze, S. O. and Okoh, A. I. 2015. Characterization of a bioflocculant (MBF-UFH) produced by *Bacillus* sp. AEMREG7. *International Journal of Molecular Science, 16:* 12986-13003, doi:10.3390/ijms160612986.

[6] Luvuyo, N., Agunbiade, O., Leonard, M. and Okoh, A. I. 2011. Studies on bioflocculant production by

Methylobacterium sp. Obi isolated from a freshwater environment in South Africa. *African Journal of Microbiology Research, 5(26):* 4533-4540.

[7] Li, Z., Zhong, S., Lei, H. Y., Chen, R. W., Yu, Q. and Li, H. L. 2009. Production of a novel bioflocculant by *Bacillus licheniformis* X14 and its application to low temperature drinking water treatment. *Bioresource Technology, 100:* 3650-3656.

[8] Yang, Y., Chen, H. and Yang, Z. F. 2012. Integration of water quantity and quality in environmental flow assessment in wetlands. *Procedia Environmental Sciences, 13:* 1535-1552. Online publication date: 1-January-2012.

[9] Keskinkan, O., Goksu, M. Z. L., Basibuyuk, M. and Forster, C. F. 2004. Heavy metal adsorption properties of a submerged aquatic plant (*Ceratophyllum demersum*). *Bioresource Technology, 92(2):* 197-200.

[10] Mamba, B. B., Dlamini, N. P. and Mulaba-Bafubi, A. F. 2009. *Acidithiobacillus caldus, Leptospirillum* spp., *Ferroplasma* spp. *and Sulphobacillus* spp. mixed strains for use in cobalt and copper removal from water. *Water South Africa 35(3):* 301-306.

[11] Salehizadeh, H. and Shojaosadati, S. A. 2002. Isolation and characterization of a bioflocculant produced by *Bacillus firmus. Biotechnology Letter, 24:* 35-40.

[12] Deng, B., Bai, B. and Hu, M. 2003. Characteristics of a bioflocculant produced by *Bacillus mucilaginosus* and its use in starch wastewater treatment. *Applied Microbiology and Biotechnology, 60:* 588-593.

[13] Zheng, Y., Ye, Z. L., Fang, X. L., Li, Y. H. and Cai, W. M. 2008. Production and characteristics of a bioflocculant produced by *Bacillus* sp. F19. *Bioresource Technology, 99:* 7686-7691.

[14] He, N., Li, V. and Chen, J. 2004. Prodution of polygalacturonic acid bioflocculant REA-11 by *Corynebacterium glutamicum*. *Bioresource Technology, 94:* 99-105.

[15] Mona, E. M. M. 2014. Production of bioflocculant by the marine actinomycete *Nocardiopsis aegyptia* sp. nov. *Life Science Journal, 11(12):* 27-35. (ISSN: 1097-8135). http://www.lifesciencesite.com.

[16] Taniguchi, M., Kato, K., Shimauchi, A., Ping, X., Fujita, K. I., Tanaka, T., Tarui, Y. and Hirasawa, E. 2005. Physicochemical properties of cross-linked poly-ã-glutamic acid and its flocculating activity against kaolin suspension. *Journal of Bioscience and Bioengineering, 99:* 130-135.

[17] Adebami, G. and Adebayo-Tayo, B. C. 2013. Comparative effect of medium composition on bioflocculant production by microoganisms isolated from wastewater samples. *Report and Opinion, 5(2):* 46-53.

[18] Gao, J., Bao, H., Xin, M., Liu, Y., Li, Q. and Zhang, Y. 2006. Characterization of a bioflocculant from a newly isolated *Vagococcus* sp. W31. *Journal Zhejiang University Science, 7:* 186-192.

[19] Harrigan, W.F. and McCance, M.F. 1976. *Laboratory Methods in Microbiology*. Academic Press London, New York, p. 342.

[20] Adebayo-Tayo, B. C. and Adebami, G. 2014. Production and characterization of bioflocculant produced by *Bacillus clausii* NB2 isolated from wastewater samples. *Innovative Romanian Food Biotechnology, 14:* 13-25.

[21] Gregory, J. 2006. Particles in water: Property and Processes. *Journal of Scientific and Industrial Research, 57:* 680-681.

[22] Yim, J. H., Kim, S.J., Ahn, S.A.H. and Lee, H.K. 2007.Characterization of a novel biofluculant pKG03, from a marine dinoflagellate, *Gyridinium impudium* KG 03. *Bioresource Biotechnology, 98:* 361-367.

[23] Nakata, K. and Kurane, R. 1999. Production of extracellular polysaccharide bioflocculants by *klebsiella pneumoniae*. *Journal of Bioscience, Biotechnology and Biochemistry, 63:* 2064-2068.

[24] Ugbenyen, A. M., Cosa, S. I., Mabinya, L. I., Babalola, O. O., Aghdasi, F. and Okoh, A. 2012. Thermostable Bacterial Bioflocculant produced by *Cobetia* sp. isolated from Algoa Bay (South Africa). *International Journal of Environmetal Resource and Public Health, 9:* 2108-2120.

[25] Nwodo, U. U., Agunbiade, M. O., Green, E., Mabinya, L. V. and Okoh, A. I. 2012. A freshwater *Streptomyces* sp. Gansen isolated from TyumeRiver, produces a predominant extracellular glycoprotein bioflocculant. *International Journal of Molecular Science, 13:* 8679-8695.

[26] Nwodo, U. U., Agunbiade, M. O., Green, E., Nwamadi, M., Rumbold, K. and Okoh, A. I. 2013. Characterization of an exopolymeric flocculant produced by a *Brachybacterium* sp. *Materials, 6:* 1237-1254.

[27] Cosa, S., Mabinya, L. V., Olaniran, A. O., Okoh, O. O. and Okoh, A. I. 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.

[28] Zhang, C. L., Cui, Y. N. and Wang, Y. 2013. Bioflocculants produced by Gram-positive *Bacillus* xn12 and *Streptomyces* xn17 for swine wastewater application. Chemical and Biochemical Engineering, *27:* 245-250.

[29] Salehizadeh, H. and Shojaosadati, S. A. 2001. Extracellular biopolymeric flocculants: Recent trends and biotechnological importance. *Biotechnology Advances, 19:* 371-385.

[30] Zhang, Z, Q., Lin, B., Xia, S. Q., Wang, X. J. and Yang, A. M. 2007. Production and application of a novel biofloculant by multiple microorganism consortia using brewery wastewater as carbon source. *Journal of Environmental Science, 19:* 667-673.

[31] Okoh, A. I., Okoli, S. A., Nwodo, U. U. and Mabinya, L.

V. 2011. Production of bioflocculant by *Methylobacterium* sp. isolated from marine environment. *Molecules, 84:* 765-772.

[32] Shimofuruya, H., Koide, A., Shirota, K., Tsuji, T., Nakamura, M and Suzuki, J. 1996. The production of flocculating substances by *Streptomyces griseus*. *Bioscience, Biotechnology and Biochemistry, 60*: 498-500.

[33] Liu, W. J., Wang, K., Li, B. Z., Yuan, H. L. and Yang, J. S. 2010. Production and characterization of an intracellular bioflocculant by *Chryseobacterium daeguense* W6 cultured in low nutrition medium. *Bioresource Technology, 101:* 1044-1048.

[34] Mabinya, L. V., Cosa, S., Nwodo, U. and Okoh. A. I. 2012. Studies on bioflocculant production by *Arthrobacter* sp. Raats, a fresh water bacterium isolated from Tyume River, South Africa. *International Journal of Molecular Science, 13:* 1054-1065.

[35] Patil, S. V., Bathe, G.A., Patil, R. H. and Salunkea, R. 2009. Production of bioflocculant exopolysaccharide by *Bacillus subtilis. Advance Biotechnology, 58:* 15-16.

[36] Mabinya, L. V., Cosa, S., Mkwetshana, N. and Okoh, A. I. 2011. *Halomonas* sp. OKOH- a marine bacterium isolated from the bottom sediment of Algoa-Bay produces a polysaccharide bioflocculant: partial characterization and biochemical analysis of its properties. *Molecules, 16:* 4358-4370.

[37] Gong, W. X., Wang, S. G., Sun, X. F., Liu, X. W., Yue, Q. Y. and Gao, B. Y. 2008. Bioflocculant production by culture of *Serratia ficaria* and its application in wastewater treatment. *Bioresource Technology, 99:* 4668-4674.

[38] Rawhia, A. A., Mahmoud, N. E., Hend, A. A. and Zakia, A. A. E. 2014. Bioflocculants produced by bacterial isolates from Egyptian soil; Characterization and Application of Extracellular Bioflocculant and Nanoparticles for treatment of River Nile Water. *Journal of Pharmacy and Biological Sciences*. ISSN: 2319-7676. 9, Issue 5 Ver. 1 (October, 2014), pp. 103-114. www.iosrjournals.org.

[39] Xia, S. Q., Zhang, Z. Q., Wang, X. J., Yang, A. M., Chen, L., Zhao, J. F., Didier, L. and Nicole, J. R. 2008. Production and characterization of a bioflocculant by *Proteus mirabilis* TJ-1. *Bioresource Technology, 99:* 6520-6527.

[40] Kumar, C. G., Joo, H. S., Kavali, R., Choi, J. W. and Chang, C. S. 2004. Characterization of an extracellular biopolymer flocculant from a haloalkalophilic *Bacillus* isolate. *World Journal of Microbiology and Biotechnology, 20:* 837-843.

Journal of Science Research
Volume 15, 2016, ISSN 1119 7333
Citation: Ajani, T. F., Adebayo-Tayo, B. C. and Alao, S. O. Screening and the effects of production conditions on biofloculants' production by microorganisms isolated from wastewater.

Textflow Limited
Ibadan, Nigeria