Original Research Article

FLOCCULATING PROPERTIES OF A BIOFLOCCULANT PURIFIED FROM *BACILLUS SUBTILIS* ISOLATED FROM THE STREAM SEDIMENTS OF ONYEARUGBULEM MARKET, AKURE.

Abstract

Soil samples (sediments of stream, its bank and abattoir soil) were collected from Onyearugbulem market abattoir, Akure, Ondo State. Bacteria were isolated from the above soil samples by dilution and pour plate methods. Screening for best bioflocculating bacteria was also performed. Effects of metal ions (such as Mg^{2+}, Ca^{2+} and Al^{2+}), temperature and pH were also determined on flocculating activities of bioflocculant. Six bacterial isolates producing flocculating substances were isolated and the bacterium with the best flocculating property was selected. The identified bioflocculant producing bacteria are *Bacillus anthracis, B. subtilis, B. thuringiensis, B. cereus Streptomyces, griseus* and *S. somaliensis.* The best bioflocculant producing bacterium was *Bacillus subtilis* and the flocculating activity of its bioflocculant was stimulated in the presence of Mg^{2+}, $Ca^{2+}$and Al^{3+}. This bioflocculant was thermostable and retained more than 80% of its flocculating activity after being heated at 100°C for 25 minutes. It had the highest flocculating activity of 85% at pH 6 with optimum bioflocculant dosage of 0.8ml.

Key Words

Bioflocculant, Onyearugbulem stream, pour plate, metal ion, flocculating property, thermostable.

Introduction

Chemical Flocculants

Flocculation is a form of chemical reaction that involves the addition of clarifying agents such as Iron (II) sulphate, Aluminium sulphate, and Iron (II) chloride in water treatment which results in the formation of colloids (Vasantharaj *et. al.*, 2013). It can also be described as a physical and chemical process used for the removal of the visible sediments and material from water which makes it a colloidal solution. Flocculation can be carried out through agitation or by the addition of flocculating agents (Moghimipour *et al*., 2014).

Bioflocculants

Bioflocculants are microorganism-produced special natural inorganic macromolecule substances that can flocculate suspended solids, cells, colloidal solids etc (Zaki *et al.*, 2011). Several microorganisms which secrete flocculation biopolymer have been screened and isolated from activated sludge, waste water, and soil (Zaki *et al.*, 2011). Species of microorganisms that have bioflocculant producing characteristics include bacteria (such as *Bacteroidites, Bacillus* sp., *Bacillus muscilaginosus*, *Bacillus subtilis*) fungi, actinomyces and algae (*Chlamydomonas reinhardtii, Chlorella minutissima)* (*Arcobacter*, *Cellulosimicrobium Cellulans, Aeromobacter xylosidans,*) (Okaiyeto *et al.*, 2013). Bioflocculants stand out among others as they have the advantage of innocuousness, biocompatibility, biodegradability and environmental friendliness, unlike organic and inorganic flocculants which are toxic and whose degradation intermediates are difficult to remove from the environment (Okaiyeto *et al*, 2015). Besides, organic flocculants such as polyacrylamide and polyethylene imine derivatives have been implicated in adverse human health effects. (Nwodo *et al*., 2014). Conversely, the enormous advantages associated with bioflocculants motivate its consideration as an alternative, hence the vast interest in the scientific and industrial community worldwide (Nwodo *et al*., 2012).

Materials and Methods

Description of study area

Onyearugbulem abattoir was selected as the study area because of its location in the large expanse of built up area comprising of low medium and high income earners with residential buildings in the north by office complexes and west and east by private schools and ships. The abattoir is about 50 metres off the express (Ilesha-Owo) and covers about 1000m^2 land mass.

Sample Collection

Soil sample from the Onyearugbulem abattoir slaughtering site and stream bank, was collected with the aid of an auger. The soil was dug in a V-shape to a depth of about 0-5cm, thin slices of the soil was removed from the sides and transferred into a clean container. With the aid of an auger, composite sediment was taken upstream where it was maximally free from contaminants (Nontembiso *et al*, 2011). Well water was collected with the aid of a sterilized fetching bucket into a clean 50litre container. Stream water was collected at three different points (upstream, mid-stream and downstream) with a clean bowl into a clean 50litre container. Abattoir waste effluent was collected directly from abattoir drainage into a clean 50litre container.

Determination of Physicochemical Properties of the Soil and Water Samples

The physicochemical properties of the soil samples used for the isolation of bioflocculant producing bacteria were determined according to Association of Analytical Chemists (A. O. A. C, 1990 and Carter 1992). Collected water samples were subjected to chemical analysis such as

Dissolved oxygen, pH, Electrical conductivity, Total Dissolved Solids, Chloride content, Nitrate, Phosphate, Magnesium content and total hardness before their respective treatment.

Determination of the pH of Soil Samples

Twenty grams of each soil sample was weighed and put in a 100 ml beaker. Twenty millilitres of distilled water was added to the sample. The suspension was left for 2 minutes, with occasional stirring using glass rod in order to enable it reach equilibrium. The pH of the suspension was determined using a pH meter (AOAC, 1990).

Determination of exchangeable magnesium and calcium component of soil sample using EDTA titration method

One litre standard flask was filled to the half mark with distilled water and 60 ml concentrated acetic acid and 70 ml ammonia solution was added. The mixture was shaken together and was made up to 1 liter mark with distilled water. This was left to stand overnight. A 10g quantity of soil sample was weighed into beaker and 100 ml ammonium acetate was added and the mixture was stirred and allowed to stand for 1 hour. The mixture was then filtered using Whattman filter paper. The filtrate was collected and bottled. A 50 ml burette, which previously had been washed and dried was filled to the level mark with 0.01M EDTA solutions. The filled burette was placed vertically on a retort stand. 10 ml of the prepared soil sample solution was then pipette and transferred into 250 ml conical flask. Five drops of 2% KCN was then added. A 7 ml volume of concentrated ammonium solution was added. Three drops of Eriochrome Black T indicator was then added and a wine red colour was obtained. The titration was repeated and the mean values were calculated. The results obtained for both Ca^{2+} and Mg^{2+} ions present together in the sample solution. To obtain Ca^{2+} alone, 10ml of the sample was pipette into a

250ml conical flask. Five drops of 2% KCN, 5 drops of 5% hydroxyl ammonium chloride and 5ml 20% KOH solution were added respectively. A pinch of calcium indicator was added to the mixture. The resulting solution was then titrated with 0.01M EDTA solution. The titration was repeated and the mean value was found. To determine Mg^{2+} ions in the solution, the value obtained for Ca^{2+} was subtracted from the total value obtained for Ca^{2+}/Mg^{2+} (AOAC, 1990).

Calculation:

% Calcium (Ca) =

% Magnesium (Mg)

$(Ca^{2+}/ Mg^{2+}) - Ca^{2+} = Mg^{2+}$

T = Titre value obtained from EDTA titration

M = Molarity of acid used

V_1= Total volume of initial extracting solution

V_2 = Volume of extracted solution used

W = Weight of soil sample

40 = Atomic weight of calcium

24 = Atomic weight of magnesium

Analysis of Particle size of Soil Samples

This was done using the hydrometer method. Those soil particles (Coarse fragment) that did not pass through the 2mm sieve were weighed and reported as a percentage of the whole weight. 50g of the fine earth fraction (greater than 2mm fraction) were put in a beaker and 100ml of 5% dispersing agent, sodium haxametaphosphate, added. The suspension was mixed with a stirring rod and allowed to soak for 30 minutes before transferring it to the bottled cup. The bottle cup was attached to the stirrer and stirred for three minutes to ensure breakage of soil aggregates. The suspension was poured into cylinder and made up to mark, stirred and both hydrometer and thermometer were inserted at specified time intervals to take readings (40 seconds for silt and clay reading and end of two hour for clay).

The formula below was used in their calculation

a. For an increase in temperature above 20°C

b. For a decrease in temperature

Where X = hydrometer reading at specified time

Y= differences between hydrometer calibrated temperature and the temperature of the solution at specified time.

W = weight of the fine earth fraction used.

Percent silt was derived by subtracting the calculated percent clay from that of silt and clay subtracting percent silt and clay from 100 gives the percent sand. From the texture triangle diagram, percent clay, silt and sand were used to draw lines parallel to the bottom, left side and right side of the triangle respectively. The area in which these lines intersect gives the class name or texture of the soil. Where the intersecting lines fall on the line between two textual names. The name of the finer fraction was used. The suspension was decanted and the process repeated until the supernatant became clear. The sand fraction was transferred quantitatively into a small beaker and dried in oven at a temperature of 105°C; it was cooled in a dessicator after which the sand fraction was weighed. It was passed through 0.2mm- 0.02mm sieve and the coarse fraction remaining on the sieve was also weighted. The total sand weight minus the coarse and fraction weight gives the fine sand fraction weight. They were expressed in percentage.

Percent clay: (% clay) = corrected hydrometer reading at 6 hrs, 52 min. x 100/ wt. of sample

Percent silt: (% silt) = corrected hydrometer reading at 40 sec. x 100/ wt. of sample - % clay

Percent sand: (% sand) = 100% - % silt - % clay

Determination of available phosphorus of soil samples

Air-dried soil sample (5g) was weighed into a beaker and 35 ml of phosphorus extracting solution, NH_4Cl was measured and added to the content of the beaker. The mixture was well stirred for 5 minutes before filtered using Whatman filter paper No 1 of which 4 ml of the filtrate was pipetted into a test tube and 4 ml of ascorbic acid was also added. The resulting mixture was allowed to stand for 30minutes on a test tube rack for colour development. The color developed was blue and the procedure was repeated for the other samples. The standard was also prepared by measuring 0.5 ml of 100ppm phosphorus standards and adding 4 ml of indicator M and R solution. Twenty five millilitres of distilled water was added. The solution was transferred into another test tube. A blank was prepared by measuring 4 ml of the ascorbic acid reagent and 25 ml of distilled water into another test tube. The available soil phosphorus absorbance was read at 660nm wavelength using the corning colorimeter model 253 (AOAC, 1990).

Determination of total nitrogen content of soil samples

A gram of each of the soil samples, which previously has been ground and sieved in a 2mm wire mesh was weighed and transferred into a 500 ml micro-Kjeldahl flask and 20 ml distilled water was added, the mixture was swirled for a few minutes and was allowed to stand for about 30 minutes. A 20 ml concentrated copper oxide catalyst was added to the mixture. The flask was then transferred to a mechanical heating mantle. The heater was placed in the fume cupboard connected to the electrical outlet socket and was switched. The flask was then left to boil for about 5 hours in the fume cupboard. After the digest has been observed to be clear of H_2SO_4 fumes in the flask, the heater was then switched off. The micro-Kjedahl flask was then removed from the heater and allowed to cool. The digest was then decanted into another flask. 100 ml of distilled was then added to the content of the flask. The micro-Kjedahl flask was then attached to the distillation apparatus. 10 ml of 40% NaOH solution was added through the funnel stop cork of the distillation apparatus. A 50 ml boric acid with indicator solution was transferred into 25 ml conical flask. The flask was then placed under the condenser of the distillation apparatus. The tip of the condenser was positioned such that it was about 4mm above the surface of the boric (H_3BO_3) solution in the conical flask. The digest was then distilled by allowing hot steam pot to pass from the steam pot into the digest in the micro-Kjedahl flask, thereby causing the digest to boil and distill over into boric acid. After about 150 ml of the distillate had been collected in boric acid, the distillation was stopped. The distilled water was then titrated with 0.5m standard hydrogen cholride. The colour change at the end point was from green to pink (AOAC, 1990).

% Nitrogen

M = Molarity of acid used

T = Titre volume

V_1 = Volume of digest

V_2 = Volume of digest used

W = Weight of sample

0.014 = Multiplication factor (i.e. milligram equivalent of nitrogen in ammonia)

Determination of organic carbon content of soil samples

Five grammes of sample was placed in a ceramic crucible and then heated to between 350°C and 440°C overnight. The sample was then cooled in a desiccator and weighed.

Organic matter content x 100

All weights were corrected for moisture water content prior to organic matter content calculation. To convert the organic matter to total organic carbon content, a conversion factor of 1.724 was used based on the assumption that organic matter contains 58% organic carbon (AOAC, 1990).

Organic Carbon (g)

Determination of exchangeable sodium and potassium of the content of soil samples using Flask emission Photometry method

Ammonium acetate extracting solution was used for the extraction of Na and K in the soil sample. The procedure was observed for the exchangeable Mg and Ca preparation. A 10g of soil sample was weighed into a baker; 100 ml of the ammonium acetate solution was added. The mixture was then filtered using Whatman filter paper. The filtrate was collected, bottled and labelled. The exchangeable Na and K were determined using the flame photometer according to AOAC, 1990.

Calculation:

Sodium (Na) or Potassium

R = Reading

V = Volume of extracting solution used

D = Dilution factor

W = Weight of soil sample used

Determination of organic matter

One gramme was weighed and transferred to 250-milliliter conical flask. A 10ml of $K_2Cr_2O_7$ Pottassium hepta dichromate was added and swirled to mix. 20 ml of concentrated sulphuric acid H_2SO_4 was rapidly added, shaken and allowed to stand for 30 minutes. The mixture was diluted with 100 ml of distilled water and five drops of ferroin indicator added, it was then titrated with 0.5N ($FeSO_4$) ferrous sulphate (Carter, 1992).

A blank titration was prepared in the same was (without soil) to standardize the dichromate solution. Percent organic matter was subsequently calculated, using the formula below;

% Organic carbon

Correction Factor (CF) = 1.33

M = Normality of solution x ml of solution used

% Organic matter soil = % organic C x 1.729.

Effects of some physicochemical factors on flocculating activity

Effect of cations on flocculating activity

The effect of different cations on bioflocculant production was assessed by replacing $CaCl_2$ in the production medium with Na^+, K^+, Mg^{2+}, Mn^{2+}, Al^{3+} and Fe^{3+} using the method of Agunbiade *et al*. (2017).

Effect of pH on flocculating activity

The effect of pH on flocculating activity of bioflocculant produced was assessed by adjusting the pH of the production medium using 0.1 M HCL and 0.1 NaOH at the pH range of 3-12 (Elkady *et al*., 2011).

Effect of temperature on flocculating activity

Heat stability was evaluated by incubating the bioflocculant solutions in water bath at a temperature range of 50, 60, 70, 80, 90 and 100^{o}C for 25 minutes. Afterwards, the residual flocculating activity was determined using the protocol of Gong *et al*., (2008) and Agunbiade *et al*., (2017).

Microbial analysis of water samples

The collected water samples were analyzed to determine microbial load and check for the presence of coliform before and after treatment (Omezuruike, 2008).

Results and Discussion

The values of organic content, organic matter, phosphorus, potassium, sodium, calcium and magnesium were higher in slaughtering site than stream bank and sediment. Also, the stream sediment is more sandy than others (Table 1). The values of sodium, potassium, iron, copper, zinc, pH, conductivity, soluble solids, dissolved solids, Total Dissolved solids, Dissolved oxygen, Biochemical oxygen demand, alkalinity, chlorine, chemical oxygen demand, hardness, sulphate and phosphate were higher in abattoir waste water than the remaining water samples. Well water had more calcium content than abattoir waste water. Also, stream water contained more lead, cadmium and cobalt than the remaining water samples (Table 2).

Escherichia coli, and *Bacillus cereus* were isolated from the three soil samples, ABSS, SBNK and STSD. Among the isolates from abattoir slaughtering site are *Citrobacter freundii* ABSS, *Bacillus subtilis* ABSS and *Monococcus luteus* ABSS which were not isolated from stream bank and sediment. However, *Staphylococcus aureus* was not isolated from abattoir slaughtering site. *Proteus mirabilis* SBNK was found in stream bank but was absent in slaughtering site and stream sediment. The following organisms were isolated from stream sediment but not found in slaughtering site and stream bank. They are *Clostridium botulinum*, *Shigella dysenteriae*, *Streptomyces somaliensis* and *Salmonella typhi* (Table 3).

Table 1 Physicochemical analysis of soil samples

Soil Sample	**ASS**	**SB**	**SS**
pH	5.16 ± 0.06^{a}	5.23 ± 0.06^{a}	5.19 ± 0.06^{a}
MC	21.55 ± 0.06^{a}	34.25 ± 0.06^{b}	5.19 ± 0.06^{c}
OC	1.55 ± 0.06^{a}	0.17 ± 0.06^{a}	0.36 ± 0.06^{b}
OM	2.67 ± 0.06^{a}	0.30 ± 0.06^{b}	0.63 ± 0.06^{c}
N	0.40 ± 0.06^{a}	0.08 ± 0.06^{a}	0.06 ± 0.06^{b}
P	32.62 ± 0.60^{a}	25.20 ± 0.06^{b}	16.52 ± 0.06^{c}
K	1.26 ± 0.06^{a}	0.49 ± 0.06^{b}	0.28 ± 0.06^{c}
Na	1.30 ± 0.01^{a}	0.73 ± 0.01^{b}	0.44 ± 0.01^{c}
Ca	2.60 ± 0.10^{a}	1.50 ± 0.01^{b}	3.00 ± 0.01^{c}
Mg	1.30 ± 0.10^{a}	0.73 ± 0.09^{a}	1.00 ± 0.06^{b}
Sand	56.80 ± 0.06^{a}	52.80 ± 0.06^{b}	80.80 ± 0.06^{c}
Clay	27.20 ± 0.58^{a}	23.20 ± 0.58^{b}	11.20 ± 0.58^{c}
Silt	16.00 ± 0.00^{a}	24.00 ± 0.00^{b}	8.00 ± 0.06^{c}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different ($P<0.05$).

KEY

pH – Hydrogen ion concentration, MC- Moisture Content OC- Organic Content, OM- Organic Matter, MC- Moisture Content, N- Nitrogen, P- PhosphorusK- PotassiumNa-SodiumCa-CalciumMg-Magnesium ASS-Abattoir Slaughtering Site, SB- Stream Bank, SS-Stream Sediments

Table 2 Mineral Analyses of selected water samples

Parameters	Well Water	Stream water	Abattoir waste water
Na	23.80 ± 0.06^{a}	32.90 ± 0.90^{b}	90.50 ± 0.12^{c}
K	13.70 ± 0.12^{a}	17.70 ± 0.12^{b}	56.50 ± 0.12^{c}
Mg	5.53 ± 0.12^{a}	5.89 ± 0.12^{b}	5.78 ± 0.12^{c}
Ca	40.20 ± 0.12^{a}	39.90 ± 0.12^{b}	11.30 ± 0.12^{c}
Pb	0.33 ± 0.12^{a}	0.42 ± 0.12^{b}	0.38 ± 0.12^{c}
Cd	0.012 ± 0.00^{a}	0.013 ± 0.00^{b}	0.010 ± 0.00^{b}
Cr	0.001 ± 0.00^{a}	0.001 ± 0.00	0.001 ± 0.00
Fe	0.31 ± 0.00^{a}	0.33 ± 0.00^{b}	0.52 ± 0.00^{c}
Cu	0.02 ± 0.00	0.05 ± 0.00	0.06 ± 0.00
Ni	0.06 ± 0.00	0.08 ± 0.00	0.07 ± 0.00
Co	0.010 ± 0.00	0.012 ± 0.00	0.009 ± 0.00
Zn	0.40 ± 0.01^{a}	0.41 ± 0.01^{a}	0.86 ± 0.01^{b}
pH	6.49 ± 0.34^{a}	6.90 ± 0.00^{a}	8.30 ± 0.12^{b}
Cond	39.80 ± 0.12^{a}	755 ± 0.00^{b}	14480 ± 0.00^{c}
SS	1.42 ± 0.12^{a}	1.59 ± 0.12^{b}	1.73 ± 0.12^{c}
DS	342 ± 0.00	377 ± 0.00	7225 ± 0.00
TDS	179.50 ± 0.12^{a}	378.72 ± 0.88^{b}	7226.73 ± 0.12^{c}
DO	5.49 ± 0.12^{a}	5.54 ± 0.1^{a}	910.41 ± 0.12^{b}
BOD	1.01 ± 0.01^{a}	1.40 ± 0.12^{b}	82.76 ± 0.01^{c}
Alkalinity	139.40 ± 0.12^{a}	320.00 ± 0.00^{b}	13600.00 ± 0.00^{c}
Chloride	73.24 ± 0.06^{a}	81.99 ± 0.00^{b}	5466.35 ± 0.12^{c}
COD	157.00 ± 0.00	168.00 ± 0.00	51200.00 ± 0.00
Hardness	189.20 ± 0.12^{a}	201.60 ± 0.12^{b}	1680.00 ± 0.00^{c}
Sulphate	223.89 ± 0.00^{a}	232.01 ± 0.00^{b}	4408.35 ± 0.12^{c}
Phosphate	11.98 ± 0.00^{a}	13.68 ± 0.00^{b}	$3967.180.00^{c}$

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different ($P<0.05$).

Table 3 Frequency of occurrence of bacteria isolated from different soils and water samples obtained from Onyearugbulem market

Suspected Organisms	WLWT	STWT	ABWW	ABSS	SBNK	STSD
1	+	+	+	+	+	+
2	-	-	-	+	-	-
3	-	+	+	-	-	+
4	-	-	-	+	+	-
5	-	-	-	+	-	-
6	+	+	+	+	-	+
7	+	+	+	-	+	+
8	-	-	-	+	+	+
9	+	-	-	+	+	-
10	-	-	-	-	-	+
11	-	+	+	-	-	+
12	-	-	-	-	-	+
13	-	+	+	-	-	+
14	+	-	+	+	-	+
15	-	-	-	+	-	-
16	-	-	-	+	+	-
17	-	-	-	+	+	-

Keys

ABSS- Abattoir slaughter site WLWT: well water STWT: stream water SBNK; stream bank ABWW: abattoir waste water STSD: streamsediments+: isolated or present -: Not isolated or present

1. *Escherichia coli* 2.*Citrobacter freundii* 3.*Bacillus subtilis* 4.*Bacillus cereus* 5.*Micrococcus luteus* 6.*Klebsiella* 7.*Staphylococcus aureus* 8.*Pseudomonas borealis* 9.*Proteus mirabilis* 10.*Clostridium botulinum* 11.*Shigella dysenteriae* 12.*Streptomyces somaliensis* 13.*Salmonella typhi* 14. *Enterobacteraerogenes* 15.*Streptomyces griseus* *16. Bacillus thuringiensis 17 Bacillus anthracis*

Six bacteria with bioflocculating potentials were isolated. They are *Bacillus cereus*, *Streptomyces somaliensis*, *Streptomyces griseus*, *Bacillus thurigiensis*, *Bacillus subtilis*[a] and *Bacillus subtilis*[b]. *Bacillus cereus* had its flocculating activity increase progressively with time, the same was recorded for *Streptomyces somaliensis*, *Streptomyces griseus and Bacillus thurigiensis*. *Bacillus subtilis*[a] and *Bacillus subtilis*[b] had their highest flocculating activities at 144hours of production and least flocculating activity at 216 hours. *Bacillus subtilis*[b] had the highest flocculating activity at all times of production. This makes it the bacterium with the best flocculating activity of all the isolates (Figure1).

Flocculating activity increased progressively with increased dosage till it attained 90% at 0.8mg/ml dosage level which is the highest. Thereafter, a progressive decline in the flocculating activities with increased dosage level was recorded (Figure 2).

Na^{2+} had the least effect on the flocculating activity of the bioflocculant followed by K^{+}. Mg2+ had the best stimulatory effect on the flocculsating activity of the bioflocculant produced from *Bacillus subtilis*[b] (Figure 3).

Least flocculating activity was recorded at pH 4 which progressively increased to a peak of 80% flocculating activity at pH8 and steadily decreased with increased pH (Figure 4).

The temperature retaining ability of the bioflocculant shown in figure 5 indicated progressive increase in temperature. Highest flocculating activity was 80% at 100°C and least was 75% at 50°C.

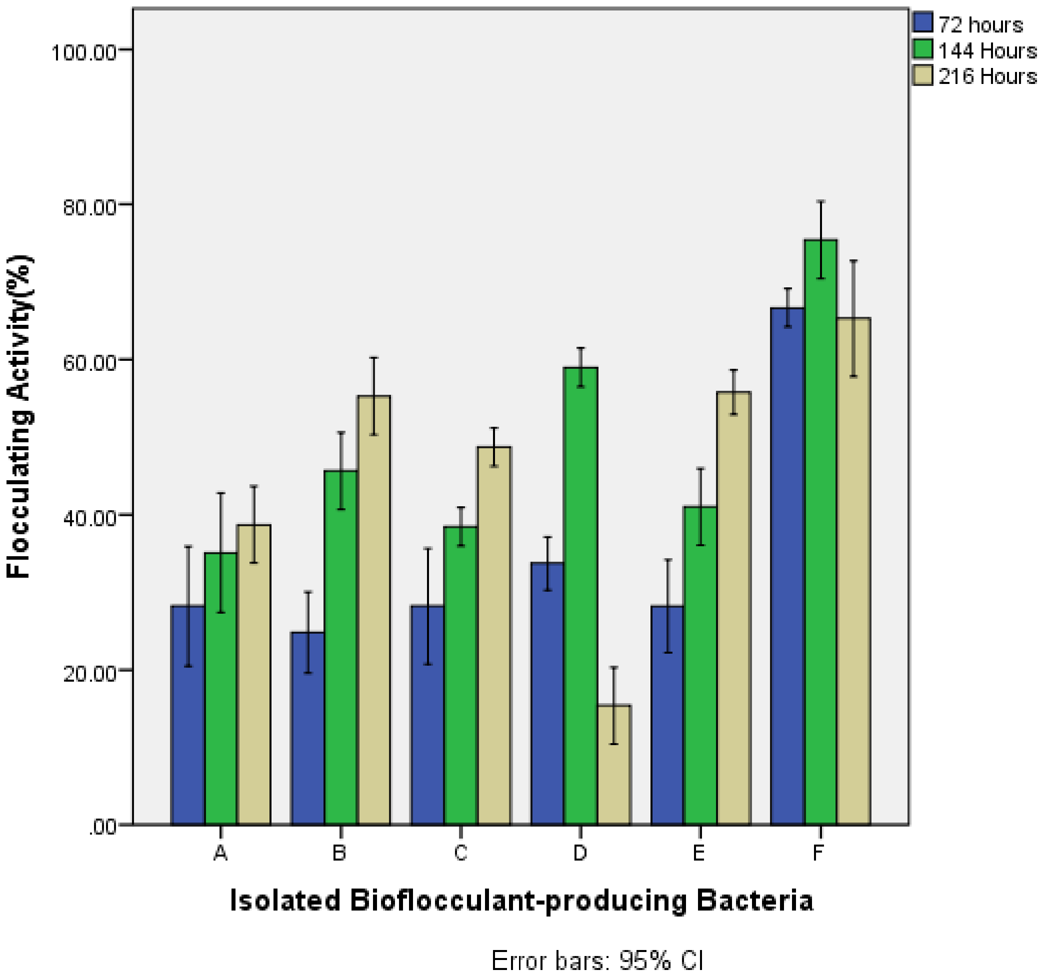

Figure 1 Flocculating activities of isolated Bioflocculant producing bacteria

KEY

A ***Bacillus cereus*** **B** ***Streptomyces somaliensis*** **C** ***Streptomyces griseus***

D ***Bacillus thuringiensis*** **E** ***Bacillus anthracis*** **F** ***Bacillus subtillis***

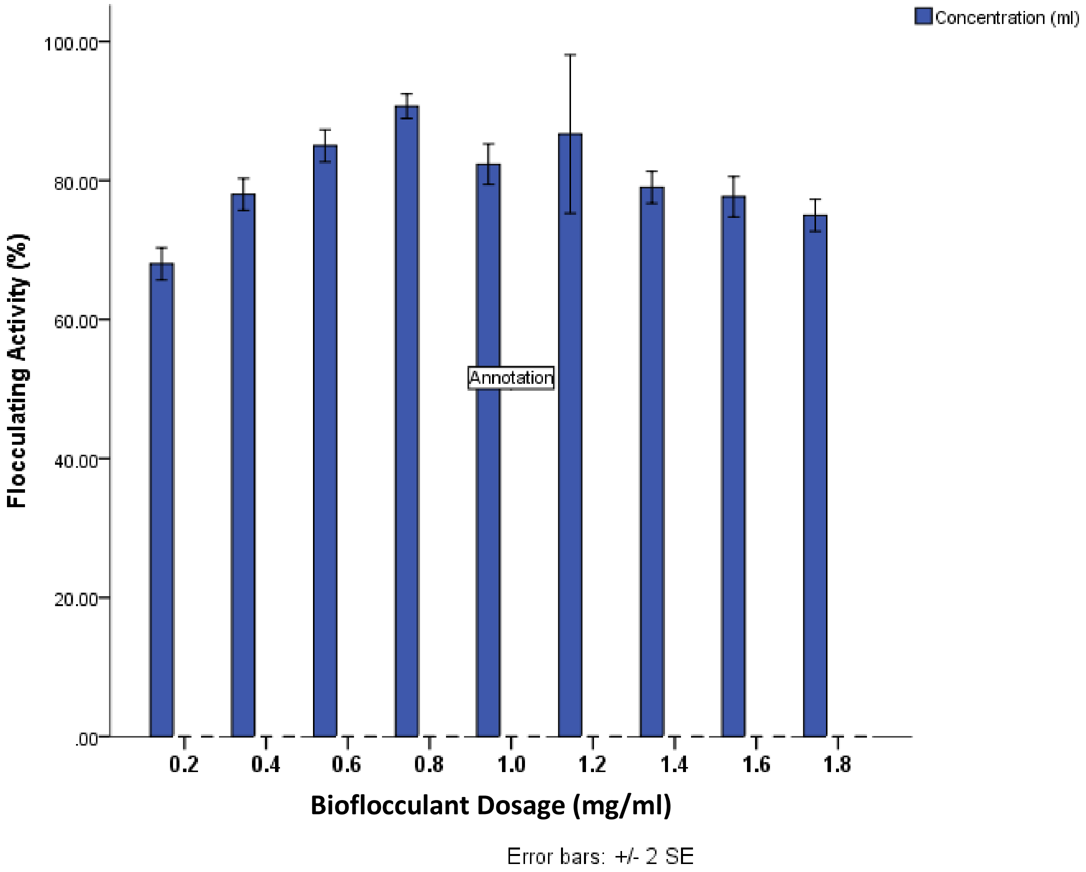

Figure 2 **Effect of Treatment dosage on flocculating activity of the bioflocculant purified from *Bacillus subtilis***

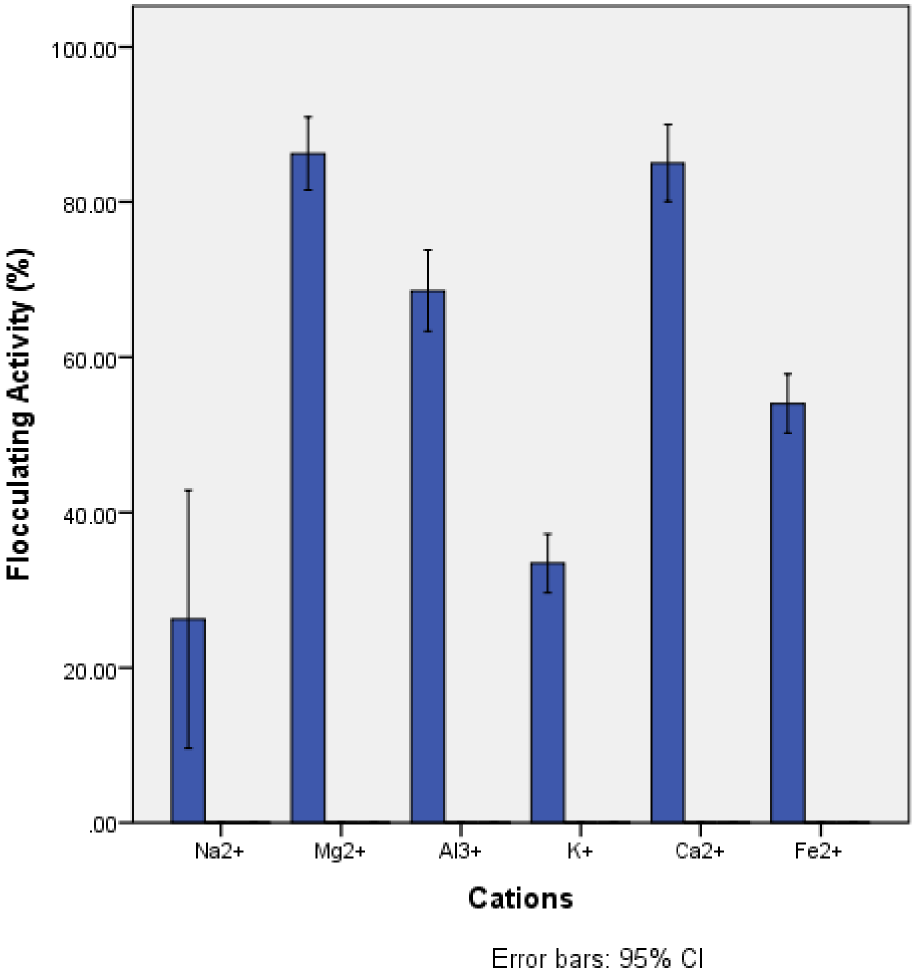

Figure 3 **Effect of cations on flocculating activity of the bioflocculant purified from *Bacillus subtillis***

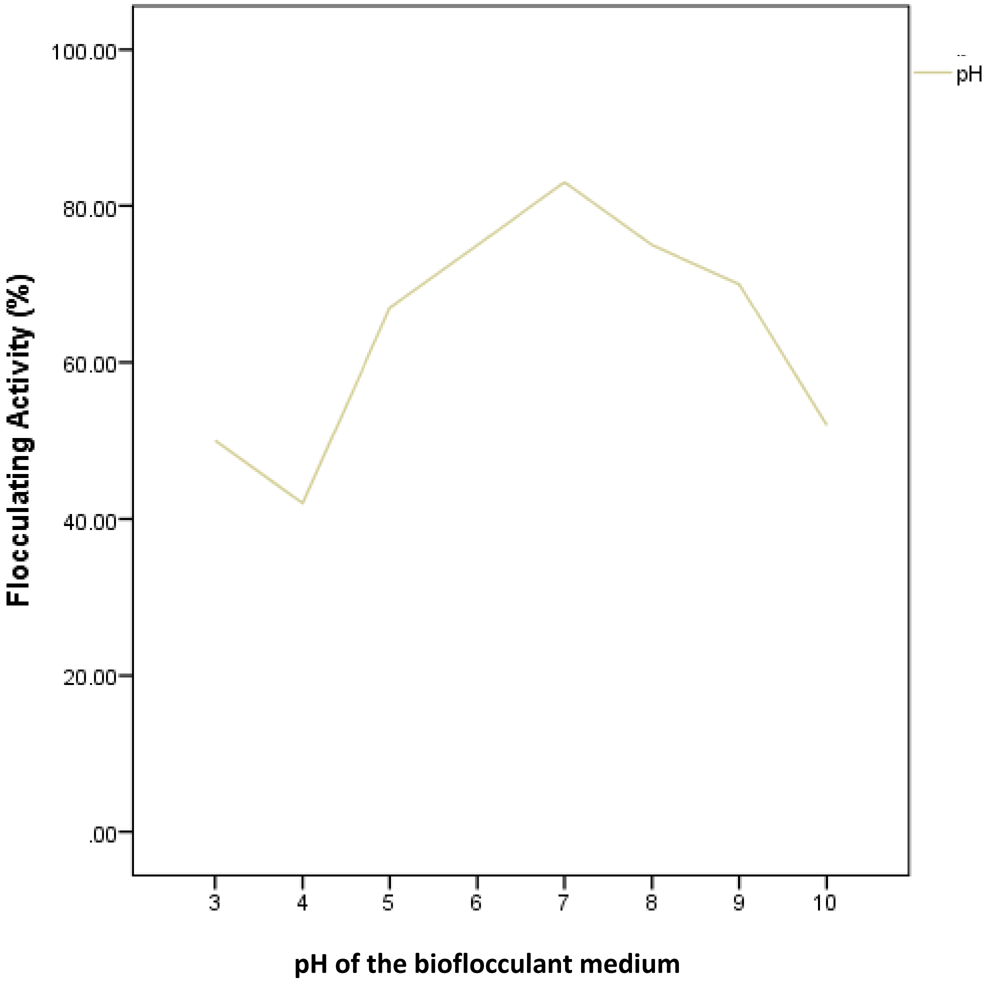

Figure 4 Effect of pH on flocculating activity of the purified bioflocculant from *Bacillus subtilis*

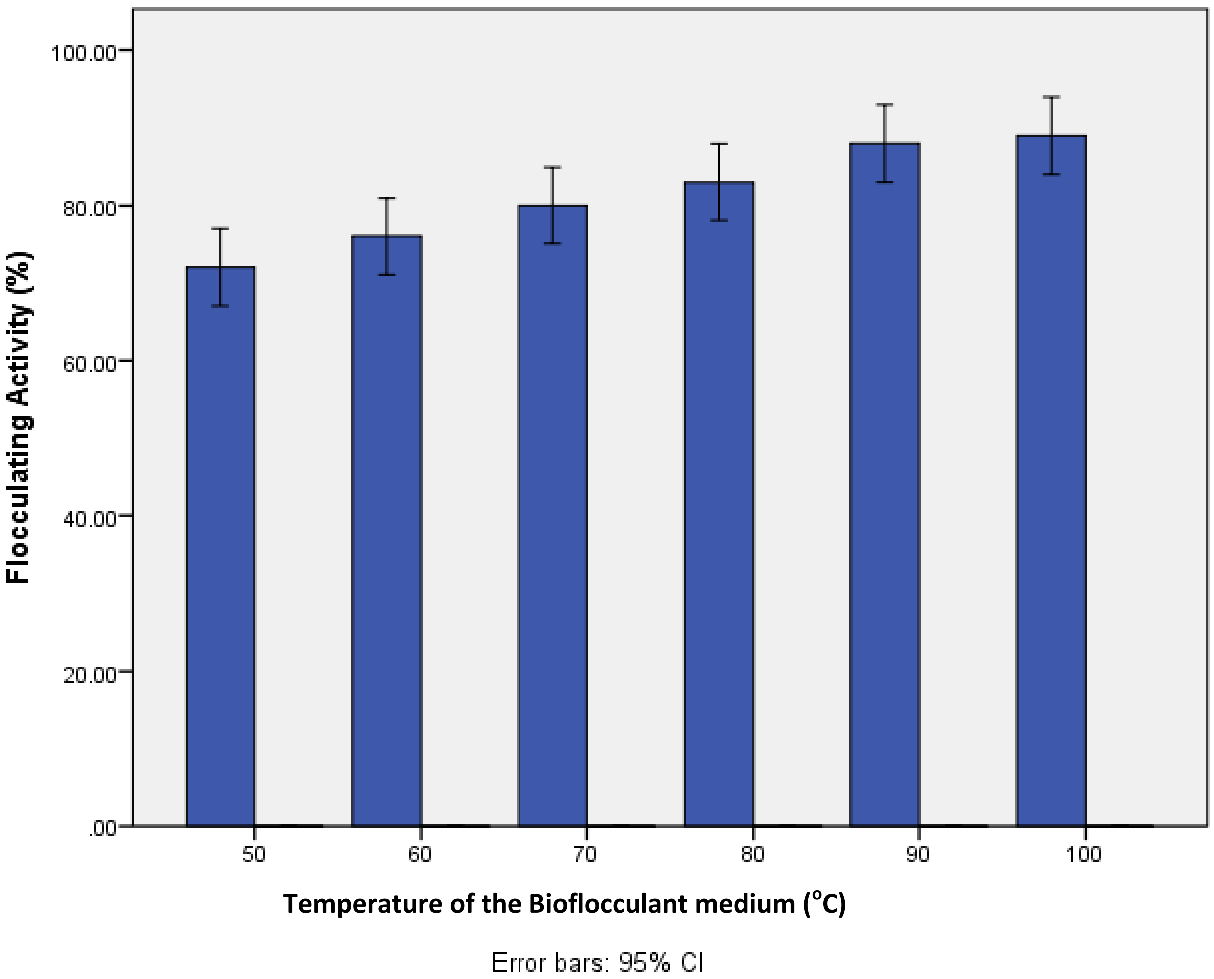

Fig. 5. Graphical representation of temperature retaining ability of the bioflocculant during progressive increase in temperature

The increased value of Abattoir Slaughtering Site than Stream Bank and Stream Sediment suggests the impact of abattoir waste on the surrounding soil. Abubakar and Tukur (2014) revealed that the discharge of abattoir effluent to the surrounding soil had significant effect on some soil chemical properties. Going by the findings of Chukwu and Anuchi (2016), the values obtained above are typical for soil samples located within the vicinity of a slaughter house. It can be deduced from the physicochemical parameters that the pH values of the soil samples were below average, indicating that the soil samples were slightly acidic (Chukwu and Anuchi, 2016). There's a possibility of contamination of the soil samples from abattoir activities (Neboh *et al*., 2013).

Well water samples consist of expected compositions of minerals for typical well water. This can be attributed to the fact that the well water is not located within the vicinity of the abattoir slaughtering site. This water sample serves as a form of control to other water samples. Mineral composition ot the stream water is a little above average and this can be associated with the fact that the utensils, containers and bodies of the slaughter men are washed in the stream where the water sample was collected (Adelegan, 2002). The mineral composition of abattoir waste water was extremely high and can be associated with the deposition of fat contents, animal wastes e.t.c, in the abattoir waste water (Teekenah *et al.,* 2012).

Bioflocculant purified from *Streptomyces griseus* (Shimofuruya *et al.,* 1996) had its flocculating substances released into the broth culture at the death phase. In this research, highest flocculating activity was achieved at 216 hours of cultivation indicating the production of more bioflocculant at the death phase. The bioflocculant purified from *Bacillus* sp 1-450 was produced during the log phase as reported by Kumar *et al* (2004). *Bacillus* sp isolated in this research had their bioflocculants produced in less than 72 hours of production which increased with time for all at 144 hours but decreased after 216 hours of production in the case of *Bacillus thuringiensis* and *B.* s*ubtilis*.

Effect of treatment dosage or inoculum size on the flocculating activity of the purified bioflocculant from *Bacillus subtilis* shows that high flocculating activity of 80% and above was achieved with treatment dosages of 0.8, 0.6, 1.0, and 1.2 mg/ml. At dosage 0.2 mg/ml, the flocculating activity was about 70%; which has the lowest flocculating activity. It can be deduced that the bioflocculant gave its best flocculation at different dosage levels of 0.6, 0.8 and 1.2. Previous studies have shown that inoculum size play important role in cell growth and bioflocculant production (Okaiyeto *et al.*, 2015). Small size inoculum prolong the lag phase, while large inoculum make niches of strain overlap excessively and consequently inhibit bioflocculant production (Okaiyeto *et al.*, 2016, Zhang *et al*., 2007). The quantity of the bioflocculant taken at varying quantities per milliliter reflects the ability of that bioflocculant to bioflocculate (Okaiyeto *et al.*, 2016). The biopolymer purified from *Bacillus mojavensis* at a dosage level of 5.2g/l attained very fast sedimentation (Elkady *et* al., 2011). Bioflocculant purified from *Bacillus* sp DP 152 at a dosage of 1mg/l brought about flocculation (Suh *et al*., 1991).

The effect of cations on the flocculating activity of the bioflocculant produced by *Bacillus subtilis* showed that except Na^+ and K^+, which drastically reduced the bioflocculating efficiency of *Bacillus subtilis*, virtually all the metal ions stimulated flocculating activity of the bioflocculant to a level above 50%. This result is in tandem with that of Ugbeyen and Okoh (2014), Okaiyeto *et al*., (2015) where the monovalent ions used completely inhibited the flocculating activity of the bioflocculant used. The variation in the flocculating activity recorded could be as a result of the fact that the bioflocculants were produced from different organisms and this therefore justifies the disparity in the effect Na+ and K+ has on the bioflocculating activity. Divalent cations were observed to better stimulate the flocculating activity of the bioflocculant produced by *Bacillus subtilis*. Ugbeyen and Okoh, (2014) had similar finding that divalent cations have good stimulatory effect on bioflocculating activities of bioflocculants. The bioflocculant showed optimum flocculating activity with Mg^{2+} and Ca^{2+} when compared with Al^{3+} and $Fe^{3+.}$ Bioflocculant produced by *Bacillus licheniformis* (CRC 10826) in an aerobic culture medium with citric acid, glutamic acid and glycerol as carbon source had its flocculating activity stimulated by $Ca^{2+.}$ Fe^{3+} and Al^{3+} with a neutral pH (Shih, 2001). *Bacillus* sp Gilbert had its source from Algoa Bay used Mg^{2+} as cation at pH 6.2 (Nontembiso *et al.*, 2011).

Studies have shown that the initial pH of the growth medium required for bioflocculant production varies from one microorganism to the other (Li-Fan and Cheng, 2010; Ugbenyen *et al.*, 2012). Xia *et* al (2008) revealed that the initial pH of the growth medium affects the electric charge of the cell and the redox reaction which in turn affect the nutrient assimilation and enzymatic reaction. The effect of pH on flocculating activity of purified bioflocculant from *Bacillus subtilis* was assessed at concentration of 0.8mg/ml with the pH of the solution ranging from 3-12. The bioflocculant flocculated a kaolin suspension with over a wide range of pH between 3 and 12 at rates above 50% except at pH 4 which is drastically low and is about 20%. From this study, it was observed that bioflocculant production is possible in almost all the pH conditions except pH 4. Optimum bioflocculant production was observed in pH 6 at 65% followed by pH 9 at 60%. This gives an impression that bioflocculant production from *Bacillus subtilis* is possible under weak acidic and alkaline conditions. This finding is similar to that of Liu *et al.* (2010) and Zulkeflee *et al.*(2012). Zheng *et al.*, (2008) and Okaiyeto *et al.*, (2015) revealed that an alkaline pH range of 7-12 was more suitable for biofloculant production of

Bacillus sp F19 with its maximum flocculating activity observed at pH 9; bioflocculant production was however inhibited under acidic conditions. The case was however different for bioflocculant produced from *Cobetia sp.*, with its optimum production at pH 6 (Ugbenyen *et al*., 2012).

Effect of temperature on the purified bioflocculant from *Bacillus subtilis*, shows that the bioflocculant purified from *Bacillus subtilis* is thermal stable. This was demonstrated with an increase in the flocculating activity of the bioflocculant with increased temperature. It is understood that bioflocculants rich in polysaccharides are more resistant to heat than those that are mainly composed of proteins or have lesser polysaccharide content (Xia *et al*., 2008; Okaiyeto *et al*., 2015).

Conclusion

Bacillus subtilis isolated from the stream sediments of Onyearugbulem abattoir stream possessed properties capable of forming colloids as a result of flake formation in the macrocromolecule produced by it. This bioflocculant can be exploited further in water treatment.

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