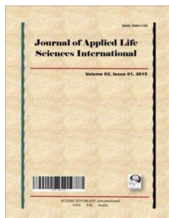

Journal of Applied Life Sciences International

14(2): 1-12, 2017; Article no.JALSI.36453
ISSN: 2394-1103

Production, Characterization and Effect of Cultural Condition on Bioflocculant Produced by *Alcaligenes aquatilis* AP4

Bukola Christianah Adebayo-Tayo[1] and Gboyega Ebenezer Adebami[1*]

[1]Department of Microbiology, University of Ibadan, Oyo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Authors BCAT and GEA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author BCAT managed the analyses of the study. Author GEA managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2017/36453
Editor(s):
(1) Renu Bharadwaj, Department of Microbiology, B. J. Govt Medical College, Pune, India.
Reviewers:
(1) Essam Hussein Abdelshakour, Al-Azhar University, Egypt.
(2) S. Sreedevi, St.Pious X Degree & PG College, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/21520

Original Research Article

Received 28th August 2017
Accepted 20th September 2017
Published 23rd October 2017

ABSTRACT

Bioflocculants are polymeric substance produced by microorganisms as a secondary metabolite which can be used in the treatment of wastewater. A novel bioflocculant producing bacterium was isolated from Palm-oil mill effluent and identified as *Alcaligenes aquatilis* AP4 using 16S rRNA gene sequencing. Production, characterization and the effect of culture conditions such as carbon source, cations, incubation temperature, initial pH, static/agitation incubation and speed on bioflocculant produced by AP4 were investigated. Glucose supported highest production of bioflocculant by the isolate at 72 hours of incubation. 30°C and pH 9.0 induced the highest bioflocculant production. Agitation condition had a significant effect ($P \leq 0.05$) while shaking speed of 140rpm induced maximum production of bioflocculant. Fourier Transform Infrared (FT-IR) spectrophotometer analysis shows the presence of OH, NH_2, $CONH_2$ CO^- and COO^- as functional groups and Chemical analyses of the purified bioflocculant revealed it to be a glycoprotein.

Keywords: Bioflocculant; Alcaligenes aquatilis; flocculating activity; palm-oil effluent; glycoprotein.

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

1. INTRODUCTION

Flocculation refers to the process by which destabilized particles conglomerate into larger aggregates so that they can be separated from the wastewater [1]. In general, flocculants are classified into three groups: inorganic flocculants, such as aluminum sulfate and polyaluminum chloride [2]; organic synthetic flocculants, such as polyacrylamide derivatives and polyethylene amine; and naturally occurring flocculants, such as chitosan, gelatin, guar gum, sodium alginate [3]. Both organic and inorganic flocculants derivatives are frequently used in both wastewater treatment and the fermentation industries because they have strong flocculating activity and are also cost-effective [4-5]. 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 [6].

A bioflocculant on the other hand, is a kind of biodegradable polymeric flocculants produced by many microorganisms during their growth [7-9]. Compared with conventional synthetic organic flocculants, bioflocculant have special advantages such as safety for ecosystems, potential flocculating effects, biodegradability and harmlessness to humans and the environment, and as a consequence may potentially be applied in drinking and wastewater treatment, downstream processing, food, pharmaceutical and fermentation processing [10-14].

Moreover, most of high molecular weight flocculants are recalcitrant. It is evident that the acrylamide monomer is not only neurotoxic and carcinogenic but also non-biodegradable in nature. They have detrimental effect both on flora and fauna [15-16]. Aluminium is one of the component of Aluminium sulphate mostly used in the treatment of raw water for household use, has been shown to cause Alzheimer's disease [17-19].

Many microorganisms, including bacteria, fungi and actinomycetes, have been reported to produce extracellular polymeric substances, such as polysaccharides, functional proteins and glycoproteins, which function as bioflocculant [3,7]. Studies carried out on the chemical composition of bioflocculant produced by *Bacillus subtilis* IFO3335 [20] *Bacillus* sp. I-471 [21] (Kumar, et al., 2004), *Bacillus subtilis* DYU1 [22], *Halomonas* sp. V3a [23], *Paenibacillus elgii* B69 [24] and *Paenibacillus mucilaginosus* [25] have shown them to be polysaccharides. *Rhodoccocus erythropolis* [26] and *Nocardia amarae* YK-1 [27] produced protein flocculants while *Arcuadendron* sp. TS-4 [28], *Klebsiella pneumonia* [29], *Bacillus clausii* NB2 [30] and *Pseudomonas aeruginosa* IASST201 [31] have been reported to produce glycoprotein bioflocculants.

The genus Alcaligenes used for this study was first classified in 1919 and since then, it has undergone several changes; [32-33]. From the literatures, Van-Trappen et al. [33] was regarded as the first to isolate and characterize *Alcaligenes aquatilis* from the sediments of the Weser Estuary, Germany, and also from a salt marsh on Shem Creek in Charleston Harbor, USA [33]. *A. aquatilis* is basically gram negative rod, catalase positive, oxidase positive, non-nitrate reducing, alpha hemolytic, citrate positive, obligate aerobe, motile with peritrichous flagella as its peculiar biochemical characteristics and it can be found in our environments such soil and water bodies [32-34].

This research aimed at production and characterization of bioflocculant produced by *A. aquatilis* AP4 which is isolated from Palm-oil mill effluent in the south-western part of Nigeria and to also determine the effect of cultural conditions on the bioflocculant production.

2. MATERIALS AND METHODS

2.1 Culture Preparation

Bioflocculant producing *A. aquatilis* AP4 culture isolated from palm oil mill effluent was collected from the culture collection of our previous work in the Department of Microbiology University of Ibadan, Ibadan Nigeria. The stock cultures were maintained on Nutrient agar, incubated at 30°C for 72hrs and stored at 4°C.

The seed culture was grown in a 250ml flask containing: Nutrient broth- 10.0 g; Potato dextrose broth, - 5.0 g; Glycerol – 3 ml; Yeast Extract - 6.5 g; Sodium chloride - 1.0 g in 1 litre of distilled water. The pH of the medium was adjusted to 7.0 and the medium was autoclaved and inoculated with pure culture from the stock culture and incubated for 24 hrs.

2.2 16SrDNA Sequence Determination and Phylogenetic Analysis of the Bioflocculant-Producing *A. aquatilis* AP4

A. aquatilis AP4 isolated from Palm oil mill effluent was identified using molecular technique based on the 16SrRNA gene amplification by polymerase chain reaction (PCR) followed by sequencing of the amplified gene as designed according to Gupta et al. [35] and Xiong et al. [36].

The isolate was incubated in 250 ml flasks containing 50 µl fresh LB medium for 16 h at 37°C with shaking at 120 rpm. The genomic DNA of the strain was then extracted using CTAB method of DNA extraction from microbes. PCR amplification was carried out to determine the partial 16S rRNA gene. The PCR program was 30 cycles of 94°C (1 min), 55°C (30 s), and 72°C (1.5 min). The PCR universal primers were 5-CCAGCAGCCGCGGTAATACG-3 (forward) and 5-TACCAGGGTATCTAATCC-3 (reverse). Purification of the PCR products and the determination of sequences were performed by Macrogen USA (9700 Great Seneca Highway, Rockville, MD 20850, USA). The 16S rRNA gene sequence of strain AP4 obtained was compared with the NCBI database [37].

2.3 Bioflocculant Production by *A. aquatilis* AP4

The isolates were used for bioflocculant production using Bioflocculant Production Broth medium (BPB). The BPB composition include: 10 g glucose, 2 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.1NaCl, 0.5 g $CaCO_3$, and 0.5 g yeast extract. The mixture was dissolved in 1 liter deionized water with the initial pH adjusted to 7.0. The medium was sterilized, inoculated with pure culture of the 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 [38].

2.4 Determination of Flocculating Activity

The flocculating activity was determined according to the method of Kurane et al. [39] as modified by Gao et al. [7]. A suspension of kaolin clay was used as test material for flocculating activity determination. The kaolin clay was suspended in distilled water at a concentration of 5 g/L at pH 7.0 and used as a stock solution for the subsequent assays. The following solutions were mixed in a test tube: kaolin clay suspension (9 mL), culture supernatant (0.1 mL) and 1% $CaCl_2$ (0.25 mL). A reference tube in which the culture supernatant was replaced with distilled water was also included and measured under the same conditions. The final volume of all mixtures was made up to 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 550 nm with a UV spectrophotometer and the flocculating activity determined as follows:

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

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

2.5 Effects of Carbon Source on Bioflocculant Production by *A. aquatilis* AP4

Effect of carbon sources such as economic wastewaters: Palm-oil effluent, Abbatoir effluent, Brewery effluent and Sewage and glucose on bioflocculant production by the isolate was investigated. Kaolin assay was carried out to check the maximum flocculating activity of the isolate after which the absorbance was then read using a spectrophotometer at wavelength of 550 nm.

2.6 Effects of pH, Incubation Temperature and Static/ Agitation on Bioflocculant Production by *A. aquatilis* AP4

Effect of pH on flocculating activity of bioflocculant produced by *A. aquatilis* AP4 was determined. The initial pH of the culture media was varied between the pH range of 3–12 by adjusting with either 0.1N HCl or 0.1N NaOH [40]. The medium was sterilized, inoculated and incubated on a rotary shaker at 120 rpm and 37°C for 3 days. Kaolin assay was carried out to check the maximum flocculating activity of the isolate.

The effect of incubation temperature on bioflocculant production by *A. aquatilis* AP4 was carried out by varying the incubation temperature between 25 – 45°C. The medium was sterilized, inoculated and incubated on a rotary shaker at 120 rpm and 37°C for 3 days. Kaolin assay was

carried out to check the maximum flocculating activity of the isolate.

Effect of incubation under static and agitation conditions were investigated. After sterilization and inoculation of the culture medium, some were incubated under static condition while others were incubated under agitation. The speed of agitation was also varied between 80 – 160 rpm. Kaolin assay was carried out to check the maximum flocculating activity of the isolate after which the absorbance was then read using a spectrophotometer at wavelength of 550 nm.

2.7 Characterization of the Bioflocculant Produced by *A. aquatilis* AP4

2.7.1 Extraction and purification of the bioflocculant

The purification and characterization of the bioflocculant was performed using the method described by Chang et al. [41] and Chen et al. [42]. Fermentation culture was prepared based on the optimal culture conditions determined earlier. After three days of cultivation, the culture was centrifuged at 4,600 rpm for 30 min and at 4°C to remove cells. One volume of distilled water was added to the supernatant and centrifuged again for 15 min to remove insoluble solutes. Two volumes of cold ethanol were added to the supernatant, and the solution was mixed and left standing at 4°C for 12 hr. 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 mixture of chloroform and *n*-butyl alcohol (5:2 v/v) was added. After mixing, the mixture was left at room temperature for 12 hr. The upper phase was centrifuged at 3,000 x g for 15 min and the supernatant was dialyzed against distilled water. Thereafter, the dialyzate was vacuum dried to obtain a pure bioflocculant.

2.7.2 Chemical analyses of the purified bioflocculants

Total sugar content of the purified bioflocculant was determined by the phenol-sulphuric acid method using glucose as the standard solution as described by Chaplin and Kennedy [43]. Total protein content was measured by the Lowry et al. [44] method using bovine serum albumin as the standard solution. The functional groups of the bioflocculant were characterized using a Fourier transform infrared spectrophotometer (Perkin Elmer System 2000, FT-IR, England). The bioflocculant was ground with KBr salt at 25°C and pressed into a pellet for FT-IR spectroscopy over a wave number range of 4 000-370 cm^{-1}.

2.8 Statistical Analysis

The Data obtained were subjected to one-way analysis of variance (ANOVA) to determine their significance at $P \leq 0.05$. Tukey-Kramer test method was used. All data were treated in replicates, the standard deviation of the mean values was taken [45].

3. RESULTS AND DISCUSSION

Bioflocculant producing *Alcaligenes aquatilis* AP4 was isolated from palm oil mill effluent. The isolate was characterized genotypically and the bioflocculant produced by the isolate was also characterized. A BLAST (Basic Local Alignment Search Tool) analyses of the 16S rRNA gene nucleotide sequence of strain AP4 PCR amplified product showed a 97% similarity to *A. aquatilis* (accession number KT748636). The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model [46]. The tree with the highest log likelihood (-3893.6749) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 0.0000% sites). The analysis involved 15 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 702 positions in the final dataset. Evolutionary analyses were conducted in MEGA 6 [47].

The effect of different carbon source on bioflocculant production by *A. aquatilis AP4* is shown in Fig. 2. There was a significant difference ($P \leq 0.05$) in the flocculating activity of *A. aquatilis AP4* at different carbon sources. At 24, 48 and 72 hrs of incubation the flocculating activity ranged from 37.05^{e} - 77.55^{a} %, 54.80^{e} - 76.61^{a} % and 64.29^{e} - 89.58^{a} % respectively in

which the highest flocculating activity was recorded in Abattoir effluent at 24 hrs and glucose at 48 and 72 hrs of incubation respectively.

The isolate was able to produce reasonable level of bioflocculant in all the carbon sources used. Ability of glucose to support the highest bioflocculant production by *A. aquatilis AP4* is in agreement with the report of Cosa et al. [48] on *Virgibacillus* sp. Rob. Glucose has been reported as a preferred carbon source in previous studies on bioflocculant production by various microorganisms. Patil et al. [49] reported that the bioflocculant produced by *Bacillus subtilis* is enhanced by glucose and sucrose as carbon sources. In the case of *Rhodococcus erythropolis*, glucose and fructose enhance elongation of the cells and the production of the bioflocculant [50]. Cosa et al. [51] found that *Virgibacillus* sp. preferred glucose as carbon source and his finding was synonymous to the work of Liu and Chen [52] on *Penicillium* sp. HHE-P7 who recorded an increase

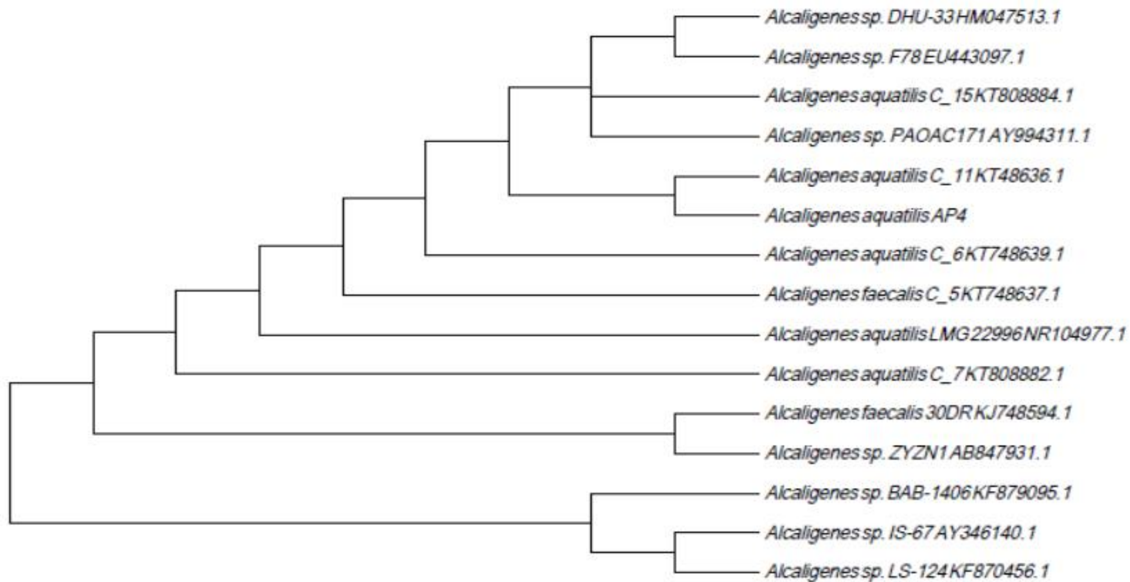

Fig. 1. Molecular Phylogenetic analysis by Maximum Likelihood method of the isolate

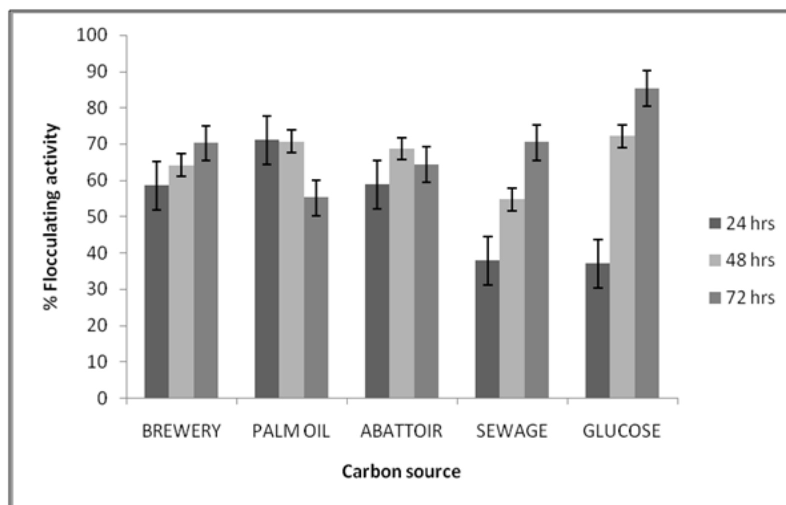

Fig. 2. Effect of different carbon source (wastewater) on bioflocculant production by *A. aquatilis* AP4

in bioflocculant production when glucose was used as carbon source. The findings of Gong et al. [53] on bioflocculant production by *Paenibacillus polymyxa* BY-28 showed that apart from glucose, sucrose, maltose, lactose and xylitol are also suitable carbon sources. However, Shih et al. [54] reported that glucose, fructose, and lactose were not suitable for bioflocculant production by *Bacillus licheniformis*, instead, simultaneous used of glutamic acid, citric acid and glycerol gave a better yield. Rasulov et al. [55] also observed that D-Mannose gave the best biomass yield and highest flocculating activity of 3.46 g/L and 97% respectively compared to glucose with 3.46 g/L and 89%.

The effect of pH on bioflocculant production by *A. aquatilis* AP4 is shown in Fig. 3. There was a significant difference (P≤0.05) in the flocculating activity of *A. aquatilis* AP4 at different pH. At 24, 48 and 72 hrs of incubation the flocculating activity ranged from 36.11^e - 71.28^a %, 30.31^e - 78.83^a % and 44.58^e - 82.94^a % in which the highest flocculating activity was recorded at pH 9.0.

This result is in agreement with the report of Salehizadeh and Shojaosadati [12] who reported that the initial pH of the production medium is one of the factors affecting the production and flocculating activity of the bioflocculant in that it determined the oxidation–reduction potential which could influence the absorption of nutrients in the production medium. The flocculating activity of bioflocculant produced by *A. aquatilis* AP4 was stable within pH 3.0 – 9.0 at 72 hrs of incubation. *Gyrodium impudicum* KG03 bioflocculant was reported to have maximum activity at acidic pH (4.0) [56]. Acidic pH was also preferred by *Aspergillus parasiticus* as reported by Deng et al. [57]. Liu et al. [58] however reported that alkaline pH 8.0 stimulated bioflocculant production by isolate *Klebsiellus* sp. TG-1. Moreover, Haas et al. [59], Patil et al. [49] and Leonard et al. [60] reported that *Corynebacterium xerosis, Bacillus subtilis* and *Arthrobacter* sp. 5J12A respectively had optimum bioflocculant production activity at neutral pH (7.0). After this pH range, a sharp decrease occurred at pH 12.0. This contradicted the report of Cosa et al. [48] on *Virgibacillus* sp. Rob who reported peak activity at alkaline pH (12.0).

The effect of incubation temperature on bioflocculant production by *A. aquatilis* AP4 is shown in Fig. 4. There was a significant difference (P≤0.05) in the flocculating activity of *A. aquatilis* AP4 at different incubation temperature. At 24, 48 and 72 hrs of incubation, the flocculating activity ranged from 41.11^e - 56.22^a %, 66.41^e - 75.55^a % and 71.11^e - 87.23^a % respectively in which the highest flocculating activity was recorded at 30°C of incubation time.

According to Zhang et al. [61], cultivation temperature have a significant impact on the enzymes responsible for bioflocculant production. Optimum temperature for maximum bioflocculant production must be known which usually varies between 25°C and 37°C [3]. Nakata and Kurane [62] reported 30°C as the optimum temperature for bioflocculant production by *Citrobacter* sp. TKF04.

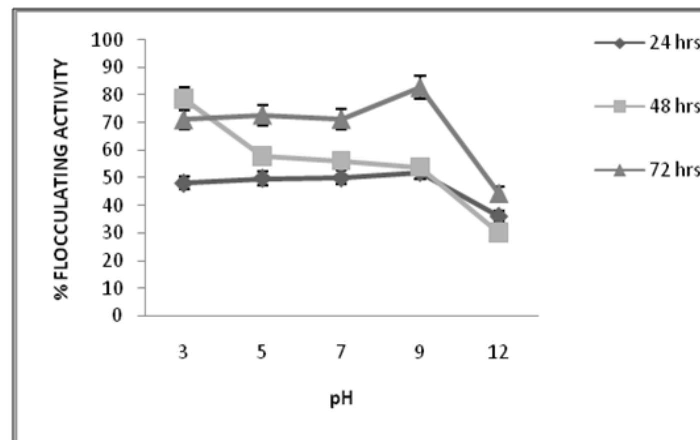

Fig. 3. Effect of pH on bioflocculant production by *A. aquatilis* AP4

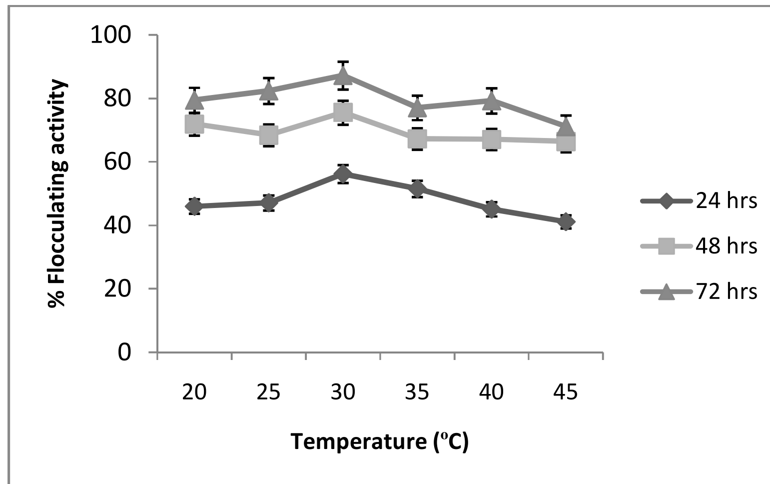

Fig. 4. Effect of incubation temperature on bioflocculant production by *A. aquatilis* AP4

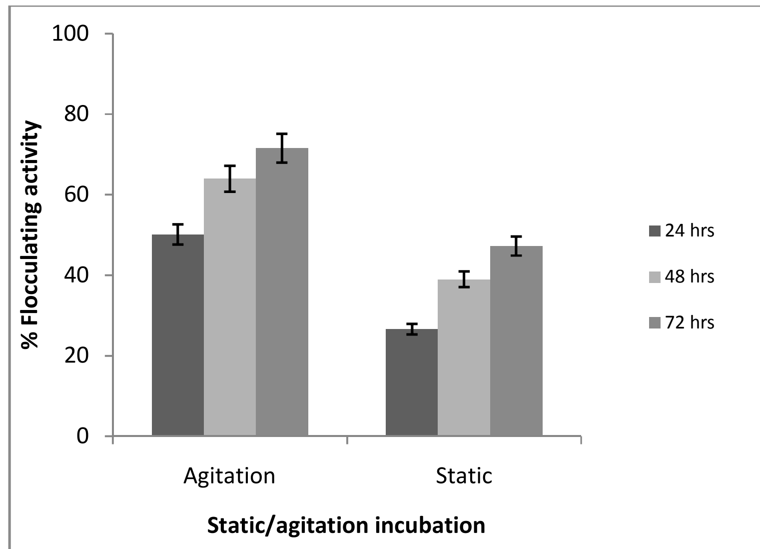

Fig. 5. Effect of static/agitation on bioflocculant production by *A. aquatilis* AP4

The effect of static/agitation on bioflocculant production by *A. aquatilis* AP4 is shown in Fig. 5. There was a significant difference (P≤0.05) in the flocculating activity of *A. aquatilis* AP4 when the incubation was carried out in static/agitation method. In static incubation, the flocculating activity ranged from 26.60^{c} - 57.25^{a}% while in agitation condition, the activity ranged from 50.15^{c} - 81.55^{a}% in which the highest flocculating activity was recorded in agitation at 72 hrs of incubation.

Maximum flocculating activity was recorded during shaking while non-shaking condition did not support bioflocculant production by the strains. This report was in contrast to what Salehizadeh and Shojaosadati [12] reported that sometimes due to agitation of the culture medium, yield of polymeric flocculant production by bacterial cells might be greatly reduced and it is therefore important to verify if there is need to agitate the culture medium. Lopez et al. [63] reported that to have increased nutrient absorption and optimum enzymatic reaction, increase in dissolved oxygen is very important and it can only be realized through the use of shaker for incubation.

The effect of speed (rpm) on bioflocculant production by *A. aquatilis* AP4 is shown in Fig. 6. There was a significant difference (P≤0.05) in the flocculating activity of *A. aquatilis* AP4 at different

incubation speed. At 24, 48 and 72 hrs of incubation the flocculating activity ranged from 54.72^{e} - 59.80^{a} %, 62.77^{d} - 73.04^{a} % and 68.92^{d} - 86.12^{a}% respectively in which the highest flocculating activity was recorded at 140 rpm speed at 72 hrs of incubation. The effect of shaking speed on the bioflocculant production showed that the shaking speed of 140 rpm was the most preferred with *A. aquatilis* AP4 having flocculating activity of 86.12%. Decrease in flocculating activity was observed when shaking below or above 140 rpm.

This may be as a result of the fact that shaking speed determines the concentration of the dissolved oxygen, which can affect the absorption of nutrients and enzymatic reaction of the strain [12]. This study contradicted the work of Zhang et al. [61] on consortium of *Staphylococcus* sp. and *Pseudomonas* sp. with 160 rpm being the best shaking speed for highest flocculating activity. Li et al. [64] also reported that agitation speed of 140–160 rpm was best for the bioflocculant produced by *Bacillus licheniformis* X14.

3.1 Biochemical and FTIR Analysis of the Bioflocculant

Chemical analysis of the purified bioflocculant produced by the isolate revealed that carbohydrate and protein are the two components present while carbohydrate happened to be the major constituent with 91.65% compared to protein with 8.35%.

The FT-IR spectroscopy performed on purified bioflocculant produced by *A. aquatilis* AP4 is shown in Fig. 7. The spectrum showed a sharp, intense absorption peak at 3421.83 cm^{-1} which is characteristic of a hydroxyl and amino group. This could be caused by the vibration of -OH or -NH in the sugar ring.

This result agreed with the report of He et al. [23] and Kavita et al. [65]. Okaiyeto et al. [66] reported that the presence of hydroxyl group was responsible for water solubility of bioflocculants. A weak peak at 2,939.61 cm^{-1} known to be typical of carbohydrates, indicated –COH asymmetrical stretching vibration. This is in accordance with the report of Yin et al. [67].

A weak peak at 2360.95 and 2320.44 is typical of aliphatic band. While that of 1869.08 – 1716.70 cm^{-1} was characterized of -C=O acid chloride. Sharp peak at 1653.05 is typical of Carboxyl, -CO-NH or $-NH_2$ group. Luo et al. [29] reported that carboxyl group provides adsorption sites for particle attachment which help the macromolecule of bioflocculant to adsorbed many particles.

Weak asymmetrical stretching peak observed from 1558.54 – 1338.64 cm^{-1} is characteristic of NH band vibration -CONH. A very sharp stretching peak at 1082.10 cm^{-1} indicated asymmetrical stretching vibration of a -C-O-C- ester linkage. Sharp peak at 885.36 cm^{-1} could be associated with glycosidic linkages between the sugar monomers.

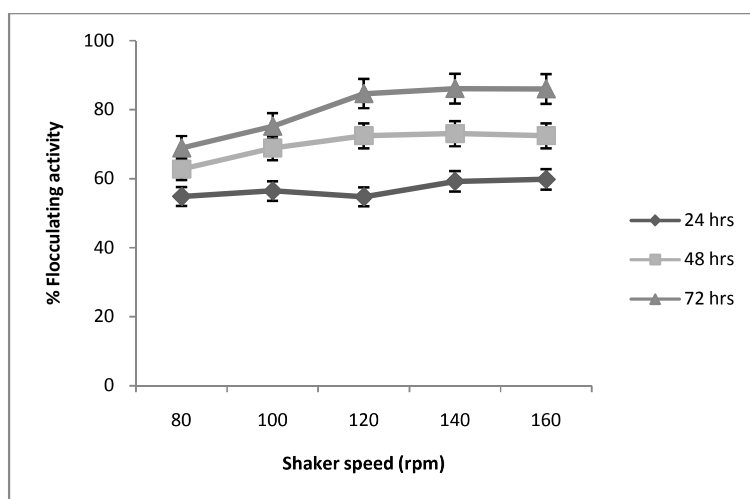

Fig. 6. Effect of speed (rpm) on bioflocculant production

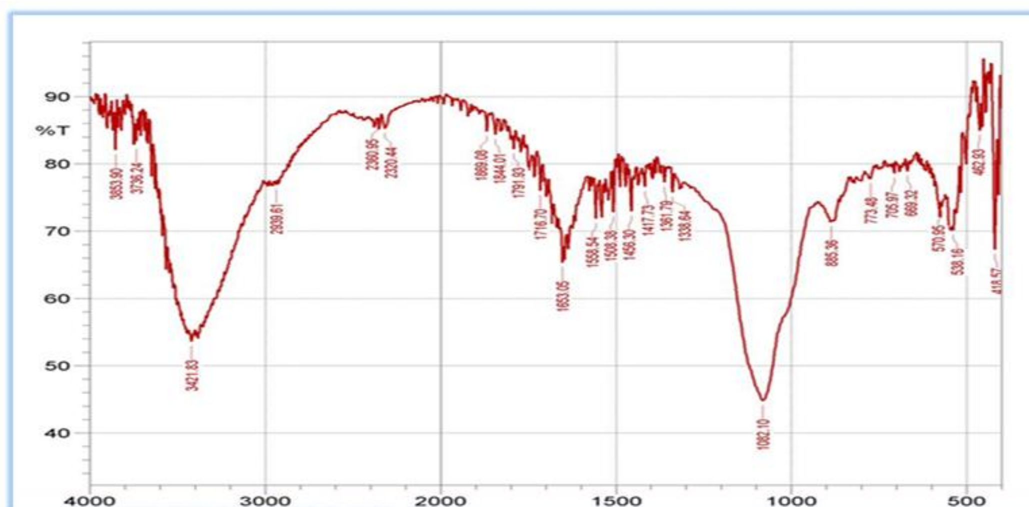

Fig. 7. FT-IR spectroscopy performed on purified bioflocculant produced by the isolate

The weak peak at 773.48 – 669.32 cm^{-1} is typical of Benzene rings. The presence of characteristic peak for carbohydrate and amide shown by infrared spectral indicated that the bioflocculant produced by *A. aquatilis* AP4 is a glycoprotein. Gao et al. [7] reported that many microorganisms such as bacteria, fungi and actinomycetes produce extracellular substances which could composed of Polysaccharide, protein and glycoprotein. Zaki et al. [68], Luo et al. [29] and Ntsangan et al. [69] reported that the bioflocculants produced by *Bacillus velezensis* 40B, *Klebsiella pneumonia and Bacillus* sp. AEMREG4 respectively are glycoprotein. However, the bioflocculants produced by *Nocardia amarae* [27] was discovered to be protein while that of *Halomonas sp.* V3a [23] and *Paenibacillus elgii* B69 [24] are polysaccharide.

4. CONCLUSION

In conclusion, *Alcaligenes aquatilis* AP4 could be regarded as novel bioflocculant producer since there is no reported history of any bioflocculant produced by this same species in the past. Cultural conditions such as carbon source, pH, incubation temperature and agitation have significant effect on the bioflocculant production. Glucose, 30°C, pH 9.0, agitation and shaking speed of 140 rpm were the best for maximum production of bioflocculant by the isolate. The bioflocculant is a glycoprotein consisting of hydroxyl, amide and carboxyl as its functional groups. Isolate AP4 is a good agent with high flocculating activity (89.58%), it therefore has the potential to be used on a large scale for bioflocculant production, which could serve as a possible substitute for non-biodegradable, carcinogenic and harmful chemical flocculants which is often used in the treatment of water today. Further studies on the biotechnological application of the bioflocculant and the genes responsible for flocculation are in progress.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Droste RL. Theory and practice of water and wastewater treatment. John Wiley and Sons, New York. 1997;384-415.
2. Lee S, John R, Mei F, Chong C. A review on application of flocculants in wastewater treatment. Proc Safety Environl Protec. 2014;92:489–508.
3. Okaiyeto K, Nwodo UU, Okoli SA, Mabinya LV, Okoh AI. Implications for public health demands alternatives to inorganic and synthetic flocculants: Bioflocculants as important candidates. Microbiol Open. 2016;5(2):177–211.
4. He N, Li Y, Chen J, Lun SY. Identification of a novel bioflocculant from a newly isolated *Corynebacterium glutamicum*. Biochem Eng J. 2002;11:137-148.
5. Suopajarvi T, Liimatainen H, Hormi O, Niinimaki J. Coagulation–flocculation treatment of municipal wastewater based on anionized nanocelluloses. Chem Eng J. 2013;231:59–67.

6. Taniguchi M, Kato K, Shimauchi A, Ping X, Fujita KI, Tanaka T et al. Phisicochemical properties of cross-linked poly-γ-glutamic acid and its flocculating activity against kaolin suspension. J Biosci Bioeng. 2005;99:130-135.
7. Gao J, Bao HY, Xin MX, Liu YX, Li Q, Zhang YF. Characterization of a bioflocculant from a newly isolated *Vagococcus* sp. W31. J Zhejiang Univ Sci B. 2006;7:186–192.
8. Defang Z, Juanjuan W, John FK. Application of a chitosan flocculant to water treatment. Carbohydr Polym. 2008; 71:135–139.
9. Mabinya LV, Cosa S, Nwodo U, Okoh AI. Studies on bioflocculant production by *Arthrobacter* sp. Raats, freshwater bacteria isolated from Tyume River. South Africa. Int J Molecul Sci. 2012;13:1054–1065.
10. Seo H. Bioflocculant production from *Bacillus* sp. A56. Sanop Misaengmul Hakhoechi. 1993;21:486-493.
11. Zhang T, Lin Z, Zhu HL. Microbial flocculant and its application in environmental protection. J Environ Sci China. 1999;11:1-12.
12. Salehizadeh H, Shojaosadati SA. Extracellular biopolymeric flocculants: recent trends and biotechnological importance. Biotechnol Adv. 2001;19:371-385.
13. Ntsaluba L, Nwodo UU, Mabinya LV, Okoh AI. Studies on bioflocculant production by a mixed culture of *Methylobacterium* sp. Obi and *Actinobacterium* sp. Mayor. BMC Biotechnol. 2013;3:1–7.
14. Ozcan E, Oner ET. Microbial production of extracellular polysaccharides from biomass sources. Springer International Publishing Switzerland. Springer International Publishing Switzerland, Polysaccharides. 2015;161-184.
DOI: 10.1007/978-3-319-16298-0_51
15. Vanhoric M, Mones W, Salehizadeh H. Carcinogen of acrylamide. Carcinogensis. 1983;4:1459 –1463.
16. Dearfield KL, Abermathy CO. Acrylamide: its metabolism developmental and reproductive effects, genotoxicity, and carcinogenicity. Mutation Res. 1988; 195:45–77.
17. Green RC, Cupple SLA, Go R, Benke KS, Edek IT, Griffith PA. Risk of dementia among white and African American relatives of patients with Alzheimer disease. J Ameri Med Asso. 2002; 287(3):329-36.
18. Kivipelto M, Ngandu T, Fratiglioni L, Viitanen M, Kåreholt I, Winblad B. Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. Archiv Neurol. 2005;62:1556–60.
19. Agunbiade MO, Heerden EV, Pohl CH, Ashafa AT. Flocculating performance of a bioflocculant produced by *Arthrobacter humicola* in sewage waste water treatment. BMC Biotechnol. 2017;17(51):1-9.
20. Yokoi H, Yoshida T, Mori S, Hirose J, Hayashi S, Takasaki Y. Biopolymer flocculant produced by an *Enterobacter* sp. Biotechnol Lett. 1996;19:569-573.
21. Kumar C, Ganesh JH, Kavali R, Choi JW, Chang CS. Characterization of an extracellular biopolymer flocculant from a haloalkalophilic *Bacillus* isolate. World J Microbiol Biotechnol. 2004;20:837–843.
22. Wu JY, Ye HF. Characterization and flocculating properties of an extracellular biopolymer produced from a *Bacillus subtilis* DYU1 isolate. Proc Biochem. 2007;42:1114-1123.
23. He J, Zou J, Shao Z, Zhang J, Liu Z, Yu Z. Characteristics and flocculating mechanism of a novel bioflocculant HBF-3 produced by deep-sea bacterium mutant *Halomonas* sp. V3a’. World J Microbiol Biotechnol. 2010;26:1135-1141.
24. Li O, Lu C, Liu A, Zhu L, Wang P, Qian C. Optimization and characterization of polysaccharide-based bioflocculant produced by *Paenibacillus elgii* B69 and its application in wastewater treatment. Bioresour Technol. 2013;134:87–93.
25. Tang J, Suijian Q, Zhigang L, Qun A, Mingquan X, Bo Y. Production, purification and application of polysaccharide-based bioflocculant by *Paenibacillus mucilaginosus.* Carbohydr Polym. 2014; 113:463–470.
26. Takeda M, Kurane R, Koizumi J. A protein bioflocculant produced by *R. erythropolis*. Agric Biol Chem. 1991;55:2663-2664.
27. Koizumi JI, Tskeda M, Kurane R, Nakamura J. Synergetic flocculation of the bioflocculant FIX extracellular produced by *Nocardia amarae*. J Gen Appl Microbiol 1991;37:447-454.
28. Lee SH, Lee SO, Jang KL, Lee TH. Microbial flocculant from *Arcuadendron* sp. TS-49. Biotechnol Lett. 1995;17:95-199.

29. Luo Z, Chen L, Chen C, Zhang W, Liu M, Han Y Zhou J. Production and characteristics of a bioflocculant by *Klebsiella pneumonia* YZ-6 isolated from human saliva. Appl Biochem Biotechnol. 2014;172:1282–1292.
30. Adebayo-Tayo BC, Adebami GE. Production and characterization of bioflocculant produced by *Bacillus clausii* NB2. Innov Rom Food Biotechnol. 2014;14:13–25.
31. Pathak M, Sarma HK, Bhattacharyya KG, Subudhi S, Bisht V, Lal B, Devi A. Characterization of a novel polymeric Bioflocculant produced from bacterial utilization of *n*-hexadecane and its application in removal of heavy metals. Frontiers Microbiol. 2017;8(170):1-15.
32. Castellani A, Chalmers AJ. Manual of tropical medicine, 3rd ed. William Wood and Co., New York; 1919.
33. Van-Trappen S, Tan T, Samyn E, Vandamme P. *Alcaligenes aquatilis* sp. nov., a novel bacterium from sediments of the Weser Estuary, Germany, and a salt marsh on Shem Creek in Charleston Harbor, USA. International J Sys Evol Microbiol. 2005;55:2571–2575.
34. Bergey DH, Breed RS, Hammer BW, Huntoon FM, Murray EGD, Harrison FC. Bergey's manual of determinative bacteriology, 4th ed., The Williams & Wilkins Co., Baltimore; 1934.
35. Gupta S, Madan RN, Bansal MC. Chemical composition of *Pinus caribaea* hemicellulose. TAPPI J. 1987;70:113–114.
36. Xiong Y, Wang Y, Yu Y, Li Q, Wang H, Chen R He N. Production and Characterization of a Novel Bioflocculant from *Bacillus licheniformis.* Appl Environ Microbiol. 2010;76(9):2778-2782.
37. National Center for Biotechnology Information. U.S. National Library of Medicine.
Available:http://blast.ncbi.nlm.nih.gov/Blast.cgi.
(Accessed 29 April, 2016)
38. Zheng Y, Ye ZL, Fang XL, Li YH, Cai WM. Production and characteristics of a bioflocculant produced by *Bacillus* sp. F19. Bioresour Technol. 2008;99:7686-7691.
39. Kurane R, Takeda K, Suzuki T. Screening for and characteristics of microbial flocculants. Agric Biol Chem. 1986; 50:2301-2307.
40. Yim JH, Kim SJ, Ahn SH, Lee HK. Characterization of novel bioflocculant, p-KG03, from a marine dinoflagellate, *Gyrodium impudicum* KG03. Bioresour Technol. 2006;98:361–367.
41. Chang WC, Soon AY, In HO, Sang HP. Characterization of an extracellular flocculating substance produced by a planktonic cyanobacterium, *Anabaena* sp. Biotechnol Lett. 1998;20(12):643-646.
42. Chen H, Zhang JF, Jiang PJ, Yang SL, Liu ZL. Composition and characterization of microbiological flocculants SC06. Environ Chem. 2002;21:360-364.
43. Chaplin MF, Kennedy JF. Carbohydrate analysis: A practical approach. Oxford, UK: IRL Press; 1986.
44. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-phenol reagent. J Biol Chem. 1951; 193:265-275.
45. Kao LS, Green CE. Analysis of Variance: Is there a difference in means and what does it mean? J Surg Res. 2008; 144(1):158–170.
46. Jukes TH, Cantor CR. Evolution of protein molecules. In Munro HN, editor, Mammalian Protein Metabolism, Academic Press, New York; 1969.
47. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30:2725-2729.
48. Cosa S, Mabinya LV, Olaniran AO, Okoh OO, Okoh AI. Bioflocculant production by *Virgibacillus* sp. Rob isolated from the bottom sediment of Algoa Bay in the Eastern Cape, South Africa. Molecules. 2011;16:2431–2442.
49. Patil SV, Bathe GA, Patil AV, Salunke BK Patil RH. Production of bioflocculant exopolysaccharide by *Bacillus subtilis.* Adv Biotechnol. 2009;8(10):14-17.
50. Kurane R, Hatakeyama S, Tsugeno H. Correlation between flocculation production and morphological changes in *Rhodococcus erythropolis* S-1. J Ferm Bioeng. 1991;72:498-500.
51. Cosa S, Ugbenyen MA, Mabinya LV, Okoh AI. Characterization of a thermostable polysaccharide bioflocculant produced by *Virgibacillus* species isolated from Algoa bay. Afr J Microbiol Res. 2013;7:2925–2938.
52. Liu L, Chen W. Characteristics and culture conditions of a bioflocculant produced by *Penicillium* sp. Biomed Environ Sci. 2010;23:213–218.

53. Gong X, Luan Z, Pei Y, Wang S. Culture conditions for flocculant production by *Paenibacillus polymyxa* BY-28. J Environ Sci and Health Part A-Toxic/Hazard Subs Environl Eng. 2003;38:657–663.
54. Shih IL, Van YT, Yeh LC, Lin HG, Chang YN. Production of a biopolymer flocculant from *Bacillus licheniformis* and its flocculation. J Bioresour Technol. 2001; 78:267-272.
55. Rasulov BA, Li L, Liu Y, Mohamad OA, Xiao M, Ma J, Li W. Production, characterization and structural modification of exopolysaccharide-based bioflocculant by *Rhizobium radiobacter* SZ4S7S14 and media optimization. Biotech. 2017;7(179):1-9.
56. Yim JH, Kim SJ, Ahn SH, Lee HK. Characterization of a novel bioflocculant, p-KG03, from a marine dinoflagellate, *Gyrodinium impudicum* KG03. Bioresour. Technol. 2007;98:361–367.
57. Deng SB, Bai RB, Hu XM, Luo Q. Characteristics of a bioflocculant produced by *Bacillus mucilaginosus* and its use in starch and wastewater treatment. Appl Microbiol Biotechnol. 2003;60:588-593.
58. Liu Z, Zhi-quan H, Tao W, Yan-ying C, Jianbin Z, Jing-ran Y. Production of novel microbial flocculants by *Klebsiella* sp. TG-1 using waste residue from the food industry and its use in defecating the trona suspension. Bioresour Technol. 2013; 139:265–271.
59. Haas SR, Nascimento FR, Schneider IH, Gaylarde C. Flocculation of fine fluorite particles with *Corynebacterium xerosis*. Revista de Microbiologia. 1999;30:225-230.
60. Leonard VM, Sekelwa C, Nwodo U, Okoh AI. Studies on bioflocculant production by *Arthrobacter* sp. Raats, a Freshwater Bacteria Isolated from Tyume River, South Africa. Int J Mol Sci. 2012;13:1054-1065.
61. Zhang ZQ, Lin B, Xia SQ, Wang XJ, Yang AM. Production and application of a novel bioflocculant by multiple microorganism consortia using brewery wastewater as carbon source. J Environ Sci. 2007; 19:667-673.
62. Nakata K, Kurane R. Production of extracellular polysaccharide biofloccculants by *Klebsiella pneumoniae.* Biosci Biotechnol Biochem. 1999;63:2064–2068.
63. Lopez E, Ramos I, Sanroman MA. Extracellular polysaccharides production by *Arthrobacter viscosus*. J Food Eng. 2003;60:463–467.
64. Li Z, Zhong S, Lei H, Chen R, Yu Q, Li H. Production of a novel bioflocculant by *Bacillus licheniformis* X14 and its application to low temperature drinking water treatment. Bioresour Technol. 2009;100:3650–3656.
65. Kavita K, Mishra A, Jha B. Extracellular polymeric substances from two biofilm forming Vibrio species: Characterization and applications. Carbohydr Polym. 2013;94:882–888.
66. Okaiyeto K, Nwodo UU, Mabinya LV, Okoh AI. Evaluation of the flocculation potential and characterization of bioflocculant produced by *Micrococcus* sp. Leo. Appl Biochem Microbiol. 2014;50:601–608.
67. Yin YJ, Tian ZM, Tang W, Li L, Song LY, McElmurry SP. Production and characterization of high efficiency bioflocculant isolated from *Klebsiella* sp. ZZ-3 Bioresour Technol. 2014;171:336–342.
68. Zaki SA, Elkady MF, Farag S, Abd-El-Haleem D. Characterization and flocculation properties of a carbohydrate bioflocculant from a newly isolated *Bacillus velezensis* 40B. J Environ Biol. 2013; 34:51–58.
69. Ntsangani N, Okaiyeto K, Nwodo UU, Olaniran AO, Mabinya LV, Okoh AI. Bioflocculation potentials of a uronic acid-containing glycoprotein produced by *Bacillus* sp. AEMREG4 isolated from Tyhume River, South Africa. Biotech. 2017;7(78):1-12.

© 2017 Adebayo-Tayo and Adebami; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21520