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# Isolation and Identification of Bioflocculants from Wastewater at Girei and Yola North, Adamawa State, Nigeria

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The aim of this study was to examine the bioflocculant-production capabilities and flocculating activities of bacteria isolated from wastewater. Wastewater samples were obtained from three disposal sites in Adamawa State, Nigeria, namely: Yola market in Yola North LGA, Vinoklang and Jaga Jaga markets in Girei LGA. The samples were diluted with distilled water, incubated for 24 h before isolating the bacteria using Pour plate method. The isolated bacterial were subjected to morphological characterization and gram staining, followed by biochemical tests to identify the probable organisms. Seven bacterial isolates: (ISO1) *Klebsiella spp,* (ISO2) *Bacillus spp.,* (ISO3) *Escherichia coli*, (ISO4) *Staphylococcus aureus*, (ISO5) *Pseudomonas aeruginosa*, (ISO6) *Salmonella spp* and (ISO7) *Proteus spp*. were identified. The organisms were then screened using bioflocculant production broth medium to ascertain their bioflocculating capabilities before determining their flocculating activity using kaolin clay suspension. Flocculating activity tests showed that four out of the seven bacteria had the ability to produce bioflocculants. The observed activities are: *Bacillus spp* (64.49%), *Klebsiella spp* (50.45%), *Staphylococcus aureus* (40.67%) and *Escherichia coli* (37.56%). The study concludes that wastewater from Yola North and Girei LGAs in Adamawa State are potential alternative sources of bioflocculants to synthetic bioflocculants.

**Keywords:** B*ioflocculant, Bacteria, Flocculating activity, Wastewater*

## 1. Introduction

In the 21st century, bioflocculants have received biotechnology and scientific considerations because of their biodegradability and the lack of secondary pollutants from their biodegraded intermediates. These have made them suitable for potential application in drinking water, fermentation process, wastewater treatment and downstream processing (Salehizadeh & Shojaosadati, 2001). Bioflocculants are natural organic macromolecular substances produced by microorganisms capable of flocculating suspended colloidal solids, cells, solids, etc. Bioflocculation is a dynamic process that results in the synthesis of extracellular polymer from living cells (He *et al.*, 2002). Different types of microorganisms such as bacteria, actinomyces, fungi and algae with capabilities to secrete flocculating biopolymer have been isolated and screened from activated wastewater and sludge (Abd-El-Haleem *et al.*, 2008). In addition, it is possible to produce bioflocculant on a very large scale economically which can be recovered from fermentation broth (Salehizadeh & Shojaosadati, 2001). In many industrial sectors, it has wide ranges of applications in areas such as microbial enhanced oil recovery, detergent, wastewater management, textile and adhesives (Abd-El-Haleem *et al.*, 2008). However, organic and inorganic flocculating agents are mostly used in fermentation industries and water treatment due to their strong flocculating properties and low cost. But then synthetic bioflocculants mostly cause environmental and health problems. For example, acrylamide monomer, a major precursor of synthetic bioflocculant polyacrylamide, has been reported to be a very strong human carcinogen, neurotoxin, recalcitrant and non degradable in nature (Dearfield *et al*., 1988; Kwon *et al.*, 1996). Presently, many countries have limited or banned the use of some of these synthetic bioflocculants such as polyacrylamide (PAM) (Xiong *et al*., 2010). On the other hand, natural bioflocculants are biodegradable and safe but have often shown weak flocculating activity during their application (Takadi & Kodowaki., 1985). Therefore, the need to explore new biodegradable bioflocculants for possible strong flocculating activity is attracting research interest especially in developing countries like Nigeria

  

(Nontembiso *et al*., 2011; Adebiyi, *et al.*, 2016; Bamanga *et al.*, 2016; Gali *et al*. 2016). This study reports the isolation and identification of bioflocculants from wastewater at Girei and Yola North Local Government Areas, Adamawa State, Nigeria.

## 2. Materials and Methods

### 2.1 Sample Collection

Sterile plastic containers were used to collect wastewater samples. The samples were transported to the Molecular Biology laboratory at Chevron Biotechnology centre at ModibboAdama University of Technology (MAUTECH), Yola, Adamawa State for refrigeration before further analysis.

### 2.2 Sterilization of Materials

All glassware was washed with morning fresh liquid wash. All work bench surface was cleaned with 70% ethanol.

### 2.3 Sample Preparation

Each sample was prepared by diluting 50 mL of the wastewater sample in 200 mL distilled water. Three-fold serial dilutions were done to the prepared sample (Cheesbrough, 2000). Pour plate method was used for inoculation in which 1 mL of diluent was pipetted and inoculated aseptically on freshly prepared nutrient agar plates. The plates were incubated at room temperature for 24 h. After incubation, morphologically distinct bacterial colonies were sub-cultured on fresh nutrient agar plates (Suryani*et al*., 2011).

### 2.4 Identification of Isolates

Morphological characteristics, gram reaction and biochemical tests were carried out in order to identify the probable organisms. The following biochemical tests were done: oxidase, urease, catalase, coagulase, citrate utilization, indole, methyl red, Voges-Proskauer test (Kirk *et al*., 1975). Each of the isolates was also tested for its ability to ferment a given sugar (lactose and sucrose) with the production of acid and gas or acid only.

### 2.5 Screening of Isolates

Screening was carried out as described by Sheng *et al*. (2006) using bioflocculant production medium. It was used to identify bacteria producing bioflocculant through the appearance on solid Yeast extract Peptone Glycerol (YPG) medium. Isolation of bioflocculant producing bacteria was carried out using agar plate containing YPG medium with composition: 20.0 g peptone,10.0 g yeast extract, 20.0 g glucose and 15.0 g agar per litre of deionized water at pH of 7. Bioflocculant producing microorganisms were originally screened based on colony morphology (mucoid, ropy). The isolated strains were grown in 50 mL of YPG medium on a rotary shaker (120 rpm) at 25$^{o}$C for 3 days and the resultant culture broth were examined for their flocculating activity. Finally, 1 strain with high stable flocculating activity for kaolin was selected for further study.

### 2.6 Determination of Flocculating Activity

The flocculating activity of each of the isolates was determined according to the method described by Gao *et al.* (2006). UV spectrophotometer (Model: UV 1200), by Shanghai on Lab Instrument Co. Ltd from China was used to measure the absorbance. Determination of flocculating activity as described by Gao *et al.* (2006) was performed as follows: A suspension of kaolin clay (4 g/L) in deionised water at pH 7 was used as stock solution for subsequent assays. The following were mixed in the test tube: kaolin clay suspension (9 mL) culture supernatant (0.1 mL) and 1% of $CaCl_2$ (0.25 mL). For the control, the culture supernatant was mixed with deionised water and measured under similar conditions. The final volume of all the mixture which includes: kaolin clay suspension (9 mL), culture supernatant (0.1 mL), $CaCl_2$ (0.25 mL) and deionized water (0.65 mL). All these make up a total volume of 10 mL. The absorbance of the clarifying upper phase solution was measured at 550nm with a UV spectrophotometer. The flocculating activity was determined as follows:

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

Where A and B stands for the absorbance at 550nm of the sample and control respectively (Gao *et al.*, 2006).

## 3. Results and Discussion

### 3.1 Results

The result presented in Table 1 shows the physical parameters of samples. The samples are distinction in colour, pH, temperature and appearance. Table 2 shows the results of a total of fourteen bacterial colonies from the 3 sets of agar plates corresponding to the three sources of waste waters. The results presented in Table 3 shows the biochemical characteristics of the isolates. The organisms identified based on their various responses to different biochemical tests were (ISO1) *Klebsiella spp*, (ISO2) *Bacillus spp*,

(ISO3) E. coli, (ISO4) *S. aureus*, (ISO5) *P. aeruginosa*, (ISO6) *Salmonella. spp* and (ISO7) *Proteus spp.* The result presented in Table 4 shows the flocculating activity of each of the four bioflocculant-producing bacteria isolates. Bacillus spp showed the highest flocculating activity (64.49%) while *E. coli* had the lowest flocculating activity (37.56%).

**Table 1.** Physical Parameters of the Wastewater Samples from Adamawa State

| Collection site | Colour | pH | Temperature (°C) | Appearance |
|---|---|---|---|---|
| Jaga jaga market | Greenish brown | 7.20 | 34 | Turbid |
| Yola market | Whitish brown | 6.40 | 30 | Clear |
| Vonoklang market | Dark brown | 7.48 | 32 | Turbid |

**Table 2.** Characterization of the Colony of Isolates in Wastewater from Adamawa State

| Water Source | Colonies | Gram reaction | Morphological Characteristics | Isolates |
|---|---|---|---|---|
| Jaga Jaga Market | COL 101 | _ | Rod shape, greenish colour | ISO 1 |
| | COL 102 | + | Bacilli in shape, whitish colony | ISO 2 |
| | COL 103 | _ | Rod Shape | ISO 3 |
| | COL 104 | _ | Cocci, yellowish-golden | ISO 4 |
| Yola Market | COL 201 | _ | Rod shape, greenish blue | ISO 1 |
| | COL 202 | + | Bacilli shape, whitish color colony | ISO 2 |
| | COL 203 | _ | Short bacilli, pigment colonies | ISO 5 |
| | COL 204 | _ | Rod short bacilli, dark colonies | ISO 6 |
| | COL 205 | _ | Thin-blue grey colony | ISO 7 |
| Vonoklang Market | COL 206 | + | Bacilli in shape, whitish colony | SO 2 |
| | COL 301 | _ | Rod shape | IS0 3 |
| | COL 302 | + | Cocci colonies yellowish-golden | ISO 4 |
| | COL 303 | _ | Rod short bacilli, dark colonies | ISO 6 |
| | COL 304 | _ | Thin-blue grey colony | ISO 7 |

-Gram negative and + Gram positive

**Table 3.** Biochemical characteristics of the Isolates

| | Isolate | | | | | | |
|---|---|---|---|---|---|---|---|
| **Test** | ISO1 | ISO2 | ISO3 | ISO4 | ISO5 | ISO6 | ISO7 |
| **Oxidase** | - | + | - | - | + | - | + |
| **Urease** | + | + | - | + | + | - | + |
| **Catalase** | - | + | + | + | + | NA | - |
| **Citrate** | + | - | - | + | NA | NA | + |
| **Coagulase** | Nr | - | + | + | + | - | + |
| **Indole** | - | - | + | - | - | - | - |
| **Methyl-red** | - | - | + | NA | - | - | + |
| **Voges-Proskeur** | + | - | - | - | - | + | + |
| **Lactose** | * | - | * | - | - | - | + |
| **Sucrose** | + | - | - | + | - | - | - |
| **Organism** | *Klebisiella spp* | *Bacillus spp* | *E. coli* | *S. aureus* | *P. aruginosa* | *Salmonella spp.* | *Proteus spp.* |

NA = Not Reactive + = Positive reaction, - = Negative reaction, * = positive and gas production.

 

**Table 4.** Flocculating Activity of the Bioflocculant-Producing Bacteria in Waste Water from Adamawa State

| Isolates | Bioflocculant-producting bacteria | Flocculating Activity (%) |
|---|---|---|
| ISO 1 | *Klebsiella spp* | 50.45 |
| ISO 2 | *Bacillus spp* | 64.49 |
| ISO 3 | *E. coli* | 37.56 |
| ISO 4 | *S. aureus* | 40.67 |

### 3.2 Discussion

In this study the bacterial isolates from the wastewater samples were identified and screened for bioflocculant production using bioflocculant production broth media. The flocculating activity of the bioflocculant-producing bacteria was carried out using kaolin clay. Four (4) out of the seven (7) isolates possess bioflocculating activity. They includes: *Klebsiella* (ISOLATE 1), *Bacillus spp* (ISOLATE 2), *Escherichia coli* (ISOLATE 3) and *Staphylococcus aureus* (ISOLATE 4). These bacteria have been known to be found in wastewater based on findings by Rabah *et al* (2008). A similar study by Mathias *et al.* (2017) from Jimeta, Adamawa state of Nigeria isolated: *Pseudomonas aeruginosa*, *Bacillus spp*, *Klebsiella spp*, *Staphylococcus aureus*, *Salmonella spp* and *Escherichia coli* from wastewater at Jimeta modern market, Gwari market and Jimeta abattoir. Again Ismite and Atuanya (2006) reported the presence of *Klebsiella spp* and *Serratia spp* among many microorganisms in raw textile effluents. According to international standards, any water contaminated to this level could pose a hazard/threat to the environment as it will be a favourable habitat for water-borne pathogens (WHO, 1996).

Wastewater in developing countries is not properly managed and is widely exposed in the environment. This poses serious public and environmental concerns. However, it has been shown by this study and others that wastewater could be a good source of bacteria with flocculating activity. Further screening and research on bacteria with bioflocculating properties is a necessary and promising venture. It could serve as an alternative to the conventional synthetic flocculants that is detrimental to public health and the environment.

*Bacillus spp* shows the highest flocculating activity (64.49%), while *Escherichia coli* has the least (37.56%). These observations and results were similar to the findings of Buthelezi *et al*. (2009) from Durban, Republic of South Africa. They used strains of *Staphylococcus aureus* (A22), *Exiguobacterium acetylicum* (D1), *Bacillus spp* (E1) and *Klebsiella terrigena* (R2) with relatively high flocculating activity to produce bioflocculant.

## 4. Conclusion

This study shows that bacteria capable of producing bioflucculant which have a potential to be used as alternative to synthetic flocculant could be isolated from wastewater found in Yola market in Yola North LGA, Jaga Jaga and Vinoklang markets both in Girei LGA in Adamawa State of Nigeria. Hence, the coherence of this study with other findings shows the accuracy and relevance of this research.

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## Conflict of interest

The authors declare no conflict of interest.

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