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Exploration of Bioflocculant-Producing Bacteria in Sokoto Metropolis Wastewater: Isolation, Characterization, and Environmental Implications

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed to isolate, identify and characterize bacteria capable of producing bioflocculants from wastewater of three (3) different refuse dump sites at Nagarta College, Gidan-Igwai, and Gidan-Dare Areas of Sokoto Metropolis using microbiological and biochemical techniques.

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Study Design: Serial dilution technique was used for the isolation of bacteria from the samples. 1ml of each of the collected wastewater samples was serially diluted from 10^{-1} to 10^{-6} into different test tubes to reduce concentration of cells.
Place and Duration of Research: This research was carried out at the laboratory of Department of Microbiology, at Usmanu Danfodiyo University Sokoto Nigeria which lasted for three months.
Sample collection: Waste water sample were collected from Nagarta College Area (A), Gidan-Igwai Area (B), and Gidan Dare-Area (C) using a sterilized syringe 10ml capacity each then transported to the Laboratory, Department of Microbiology Usmanu Danfodiyo University Sokoto Nigeria for further analysis.
Methodology: Bioflocculant-producing bacteria were isolated from wastewater through serial dilutions, inoculation, microbial count, and growth on Yeast Extract Peptone Glycerol(YPG) medium. Bioflocculant activity was assessed spectrophotometrically.
Results: Out of the seven bacteria isolated and screened for bioflocculant production, five demonstrated significant flocculating activity. These strains were further identified as various rod-shaped bacteria species, including *Bacillus sp., Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella sp., and Staphylococcus aureus*. Notably, *Klebsiella sp.* exhibited the highest flocculating activity, reaching an impressive rate of 48.55%. It was followed by *B. subtilis* with a flocculating activity of 25.21%, *P. aeruginosa* at 24.97%, *S. aureus* at 15.90%, and*Bacillus sp*. with of 1.06%.
Conclusion: This study highlights the promising potential of these identified rod-shaped bacteria species for bioflocculant production. The significant flocculating activity demonstrated by these bacteria indicates their suitability for application in wastewater treatment. The isolated bacteria could be utilized in wastewater treatment, providing benefits like environmental preservation, enabling water reuse and recycling, protecting public health, and economic advantages, leading to a safer and more sustainable community.

Keywords: Bioflocculants; wastewater treatment; bacterial isolation; characterization.

1. INTRODUCTION

Bioflocculation refers to a process in which mediation of flocculants is achieved in the presence of microorganisms or biodegradable macromolecular flocculants released by microorganisms [1]. Microorganisms like bacteria or fungi produce bioflocculants that interact with suspended particles, causing them to aggregate [2]. This aggregation simplifies the separation of solid particles from the liquid phase, facilitating processes such as wastewater treatment [2,3].

Bioflocculants are extracellular biodegradable, non-toxic and environmentally friendly substances produced by diverse microorganisms [4]. The diversity of these products plays an important role and implication for the dynamics of organic and inorganic matter in varied ecosystems [3]. Bacteria and plants are recognized sources of bioflocculants, with plants being directly employed for their bioflocculating properties [5]. Various sources which includes soil, activated sludge, and palm mill effluents, wastes water [6], have been utilized for the production of bioflocculants [6]. Bioflocculant synthesis is influenced by the type of carbon source, such as sucrose, glucose, lactose, and others {2}, [5,6]. Toxic and polluted material from the waste water must be well treated or removed before the water is release for different uses in our environment [7]. Although, separation techniques have been widely adopted in most factories to recover suspended solid materials in waste water, the process is however time consuming and the separation efficiency is low. Consequently, the need for the use of biological flocculants which appear to be gaining global acceptance over chemical flocculants [8-11]. This study was designed to isolate and characterize bioflocculant-producing bacteria from wastewater. The ultimate goal was to contribute to the development of sustainable, eco-friendly and effective bioflocculants for wastewater treatment and industrial applications.

2. MATERIALS AND METHODS

2.1 Sample Collection

Waste water samples were collected from three different refuse dump sites at Nagarta College Area (A), Gidan-Igwai Area (B), and Gidan-Dare Area (C) using a sterile syringe of 10ml capacity each, stored in sterile plastic vials then transported to the Laboratory, Department of Microbiology Usmanu Danfodiyo University Sokoto (UDUS) for further analysis.

2.2 Growth Media Composition and Bioflocculant Production

The composition of the growth media for bioflocculant production was as follows: Glucose 3.00 g, $KH_2PO_4$0.30 g, $K_2HPO_4$0.75 g, $(NH_4)_2SO_4$0.030 g, NaCl 0.015 g, Urea 007.5 g and Yeast extract 0.075 g in 150ml of deionized water with initial of pH 6.5 [12]. 5ml bacterial suspension was prepared in accordance to 0.5 McFarland standards [13]. Batch fermentations were carried out for 5days in 250 ml flasks on rotary shaker (120 rpm) at 35°C which contained 15 ml of production medium per 2 ml of bacterial suspension. The cell-free supernatants were obtained after centrifugation for 15mins at 3500 rpm to determine the flocculation activity of the products.

2.3 Isolation of Bioflocculant-Producing Bacteria

Serial dilution technique was used for the isolation of bacteria [14]. 1ml of each of the collected wastewater samples was serially diluted from 10^{-1} to 10^{-6} into different test tubes to reduce concentration of cells. Isolation of bioflocculant-producing microorganisms was carried out using an agar plate culture containing Yeast Extract Peptone Glycerol (YPG) medium according to method described by [15]. The composition of medium; peptone 2.0 g, yeast extract 1.0 g, glucose 2.0 g and agar 1.50 g per 100ml of deionized water at pH 6.5. The serially diluted wastes water samples were then pour plated on YPG media and incubated for a period of 24 hours at 37 °C for the isolation.

2.4 Screening for Bioflocculant-production

Bioflocculant-producing microorganisms were originally screened based on colony morphology [15], biochemical tests and flocculating activities [16].

2.5 Determination of Flocculating Activity

The flocculating activity was determined from the cell-free supernatants according the method described by Ugbenyen et al. [16] with modifications. Kaolin clay suspension was prepared using 4.0 g in 1.0 L of distilled water. A mixture of 9.5 ml of kaolin suspension with 0.3 ml of 1.0% calcium chloride ($CaCl_2$) solution and 2.0% (v/v) cell-free supernatant was prepared. The mixed solution was vigorously agitated and left to settle at room temperature for 5mins. The optical density (OD) of the obtained clarified solutions was determined via spectrophotometry at 550 nm. A control sample was prepared in the same way, except the cell-free supernatant was replaced with unfermented broth media. The flocculating activity was calculated using the following expression [16].

$$\text{Flocculating activity (\%)} = (A_c - B_s)/A_c \times 100$$

Where, $A_c$and $B_s$represent the Optical Density (OD) at 550 nm of the control (A_c) and real samples (B_s), respectively.

2.6 Identification of Isolates

Bioflocculant-producing microorganisms were originally identified based microbiological observations such as colony morphology in various culture media and grams staining and they were also subjected to various biochemical tests for confirmation [17]. The tests include starch hydrolysis test, triple sugar ion test, they were further tested for indole production, Methyl red test, Voges-proskauer test, citrate utilization, urease test, oxidase test, catalase tests [18-20].

3. RESULTS

3.1 Physical Parameters of Wastewater Samples Collected

The physical parameters of the water samples (Table 1) the samples were turbid and exhibited variations in pH, temperature, and colour.

3.2 Gram Staining and Characteristics of the Colony of Isolates

Table 2 shows a total of seven (7) bacterial isolates after screening on YPG medium agar plates. The colonies were sub-cultured on freshly prepared nutrient agar plates where the results of gram staining reaction and colony characteristics were reported as Rod-shaped Gram positives (A_1, A_3, and B_2) and some others as Rod-shape Gram negatives (A_2, B_1, B_3 and C).

3.3 Biochemical Tests Identification

The biochemical characteristics of the isolates (Table 3) the organisms were identified based on their various responses to different biochemical tests as (A) B*acillus sp.,* (A_2) E*nterobacter sp.*, (A_3) B*acillus subtilis* (B) P*seudomonas aeruginosa,* (B_2) B*acillus sp.,* (B_3)K*lebsiella sp.* and (C)S*taphylococcus aureus.*

Table 1. Physical parameters of the Wastewater Samples collected

Sample	Colour	Temperature	pH	Apperance
A (Nagarta Collage Area)	Whites	36	7.40	Turbid
B (GidanIgwai Area)	Dark	29	6.98	Turbid
C (Gidan Dare Area)	Off-white	32	6.42	Less turbid

Table 2. Gram staining and characteristics of the colony of isolates

Sample	Gram Staining	Morphology
A_1	+	Rod shape, Chain
A_2	-	Short Rod
A_3	+	Rod shape
B_1	-	Short Rod
B_2	+	Rod shape
B_3	-	Short Rod
C	-	Short Rod

Table 3. Biochemical tests identification

Sample	G/R	Mpg	Glu	Suc	Lac	Gas	Stch	Cat	MR	VP	Ind	Cit	OX	Ur	H_2S	Organism
A	+	Rod Chain	+	-	-	-	-	+	+	-	-	-	+	-	-	B*acillus sp.* 1
A_2	-	Short Rod	+	-	-	-	+	+	+	+	-	+	+	+	-	E*nterobacter sp.*
A_3	+	Rod	-	-	-	-	-	+	-	+	-	+	-	+	-	B*acillus subtilis*
B	-	Short Rod	+	-	-	-	+	+	+	-	-	-	-	+	-	P*seudomonas aeruginosa*
B_2	+	Rod	+	-	-	-	-	+	-	-	-	-	+	-	-	B*acillus sp.* 2
B_3	-	Shot Rod	+	-	-	-	+	+	+	-	-	-	+	+	-	K*lebsiella sp.*
C	-	Short Rod	+	-	-	-	-	+	+	-	-	+	-	+	-	S*taphylococcus aureus*

Key; + = Presence, - = Negative G/R= Gram Reaction, MPG= Morphology, GLU=Glucose, SUC=Sucrose, LAC=Lactose, STCH=Starch, CAT=Catalase, MR= Methyle Red, VP = Voges-Proskaurer, IND=Indole, CIT=Citrate, OX=Oxidase, UR=Urease, H_2S=Hydrogen sulphide.

Table 4. Flocculation activity of the bioflocculant-producing bacteria

Isolates	Bioflocculant-Producing Bacteria	Flocculating Activity (%)
A_1	B*acillus sp.* 1	1.060
A_2	E*nterobacter sp.*	ND
A_3	B*acillus subtilis*	25.21
B_1	P*seudomonas aeruginosa*	24.97
B_2	B*acillus sp.* 2	ND
B_3	K*lebsiella sp.*	48.52
C	S*taphylococcus aureus*	15.90

Key: ND = Not detected

3.4 Flocculation Activity of the Bioflocculant-producing Bacteria

The bioflocculating activity of bioflocculant-producing bacteria isolated from wastewater samples (Table 4) indicated that the flocculation activity varied among the isolates, *Klebsiella sp.* exhibited the highest flocculating activity, reaching an impressive rate of 48.55%. It was followed by *B. subtilis* with a flocculating activity of 25.21%, *P. aeruginosa* at 24.97%, *S. aureus* at 15.90%, and lastly, *Bacillus sp*. with the least effective flocculating activity of 1.06%.

4. DISCUSSION

The increasing pollution levels in wastewater present significant environmental and health challenges, particularly in developing regions such as Sokoto state in Nigeria. A viable solution to mitigate this issue lies in the utilization of bioflocculant to facilitate the agglomeration and removal of suspended particles in wastewater. In this study seven (7) bacterial strains were identified as rod-shaped bacterial strains based on morphological characteristics and biochemical tests, as B*acillus sp.* 1 (A_1), E*nterobacter sp.* (A_2), B*acillus subtilis* (A_3), P*seudomonas aeruginosa* (B_1), B*acillus sp.* 2 (B_2), K*lebsiella sp.*($_{B3}$), and S*taphylococcus aureus* (C),with three (3) strains being Gram-positive and the remaining four (4) strains Gram-negative, as determined through gram reactions. This was consistent with the studies of [16,21], which report the identification of both Gram-positive and Gram-negative bioflocculant-producing bacteria.

Flocculation activity assessed based on kaolin flocculation rates revealed, K*lebsiella sp*. as the most active with a remarkable bioflocculant activity. This finding aligns with previous studies of [21,22] reporting high flocculation activity by K*lebsiella sp*. B*acillus subtilis* strains exhibited notable positive impacts on flocculation, with different strains demonstrating varied activities. These results build on the existing evidence of [23], which identified more than 34 B*acillus* strains. Contrary to exiting studies by [24], *Bacillus sp.* 2 and E*nterobacter sp*. showed the absence of bioflocculation activity, indicating potential challenges or strain variations may be as a result of agitation of culture medium, temperature, pH and other factors affecting bioflocculant production. P*seudomonas aeruginosa* exhibited significant flocculation activity [25]. S*taphylococcus aureus* exhibited moderate flocculation activity compared to other isolates. Similarly, this was consistent with prior research findings of [26].

5. CONCLUSION

This study presents a significant opportunity for advancing wastewater treatment processes and promoting sustainable environmental practices. The research successfully identified various bacterial strains capable of producing bioflocculants, which can enhance the efficiency of wastewater treatment by facilitating the aggregation and removal of suspended solids. This study will contributes to the development of sustainable, eco-friendly and effective bioflocculants for wastewater treatment and industrial applications. The significant flocculating activity demonstrated by these bacteria indicates their suitability for application in wastewater treatment as it is less expensive and risk free as compared to chemical water treatment. Future research should focus on optimizing the production conditions of these bioflocculants for commercial applications and exploring their mechanisms of action.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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