

**Original Article****Isolation of Biofloculant Producing microorganisms from fish pond water and sediment**

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ABSTRACT

Biofloculants are biodegradable, eco-friendly alternatives to synthetic flocculants, and microorganisms from aquatic environments represent promising sources for their production. This study was conducted to isolate and characterize biofloculant-producing microorganisms from fish pond water and sediment. Water and sediment samples were collected from four fish ponds designated P1, P2, P3, and P4. Microbial enumeration was performed using standard pour plate techniques, and isolates were purified and characterized based on morphological, microscopic, and biochemical features. Bacterial colony counts ranged from 1.10×10^4 to 9.4×10^4 CFU/mL in water and 1.26×10^5 to 1.75×10^5 CFU/mL in sediment, while fungal counts ranged from 1.8×10^3 to 2.7×10^3 CFU/mL in water and 3.2×10^3 to 4.6×10^3 CFU/mL in sediment. Sediment samples consistently yielded higher microbial loads than water samples across all ponds. A total of thirty-six bacterial and twelve fungal isolates were obtained and characterized. Bacterial isolates were identified as species of *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Escherichia*, *Enterobacter*, *Micrococcus*, *Aeromonas*, *Klebsiella*, *Acinetobacter*, *Proteus*, and *Citrobacter*, with *Bacillus* and *Pseudomonas* being the most dominant genera. Fungal isolates included *Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium*, *Rhodotorula*, *Trichoderma*, *Mucor*, *Cladosporium* and yeasts. Several isolates exhibited mucoid colony morphology. The presence of known biofloculant-producing genera such as *Bacillus*, *Pseudomonas*, *Aspergillus*, *Penicillium*, and *Rhizopus* indicates that fish pond water and sediment harbour diverse microorganisms with significant biofloculant-producing potential. These findings provide a foundation for further quantitative screening, molecular identification, optimization, and application of biofloculants in the treatment of water and wastewater.

Keywords: Biofloculant, fish pond, water, sediment, microbial isolation,

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INTRODUCTION

Water is an essential natural resource for human health, domestic activities, agriculture, industry and ecosystem

sustainability. However, increasing contamination of water resources by suspended solids, organic matter, microorganisms, industrial effluents and other pollutants has created a growing

need for efficient and environmentally sustainable water-treatment technologies. Coagulation–flocculation is one of the most widely applied processes for removing suspended and colloidal particles from contaminated water because it facilitates the aggregation of small particles into larger flocs that can subsequently be separated by sedimentation or filtration [1]. Conventional treatment processes commonly depend on chemical coagulants and flocculants such as aluminium and iron salts and synthetic polymers. Although these materials can provide effective particle removal, concerns regarding chemical residues, sludge production, environmental persistence and treatment sustainability have stimulated interest in alternative flocculating agents derived from natural and biological sources [2].

Bioflocculants are biodegradable polymeric substances produced by microorganisms and are capable of promoting the aggregation and sedimentation of suspended particles in aqueous systems. They may comprise polysaccharides, proteins, glycoproteins, nucleic acids or combinations of extracellular polymeric substances (EPS) [3]. Microbial EPS are particularly important because they mediate adhesion, aggregation and interactions between microbial cells and surrounding particles. Their functional groups, including hydroxyl, carboxyl and amino groups, can interact with suspended particles and dissolved substances, thereby facilitating particle destabilisation and floc formation. Microbial bioflocculants have consequently attracted increasing attention as potential substitutes or complements to conventional chemical flocculants in water and wastewater treatment [4].

The use of microorganisms as sources of bioflocculants offers several potential advantages. Microorganisms can grow rapidly on relatively inexpensive substrates, and their metabolic products can be obtained through controlled fermentation. In addition, microbial bioflocculants are generally biodegradable and may have lower environmental impacts than some synthetic polymers [5]. Their application has been investigated for the removal of suspended solids, dyes, heavy metals, organic pollutants and other contaminants from wastewater. Recent research has further emphasised the potential of microbial flocculants to partially replace chemically synthesised flocculants while contributing to more sustainable wastewater-management practices. Nevertheless, the effectiveness of microbial bioflocculants varies considerably among microorganisms because their production, chemical composition and flocculating mechanisms are influenced by strain characteristics and environmental conditions [5,6].

Microorganisms capable of producing bioflocculants have been recovered from diverse natural and engineered environments, including river sediments, wastewater, activated sludge, soil and aquatic systems. Bacteria are particularly important in bioflocculant research, with genera such as *Bacillus*, *Pseudomonas*, *Acinetobacter* and related organisms reported to possess flocculant-producing capabilities. Fungi, yeasts and other microorganisms may also produce extracellular polymers with flocculating properties. For example, a study of bioflocculant-producing bacteria isolated from river sediment demonstrated considerable differences in flocculating activity among isolates, with a *Bacillus velezensis* strain showing

very high activity against kaolin suspension [7].

Fish-pond environments represent a potentially valuable source of bioflocculant-producing microorganisms because they contain substantial quantities of organic and particulate materials. Fish faeces, uneaten feed, algal biomass, dead microorganisms and other organic residues continuously enter and accumulate within earthen pond systems. These materials support diverse microbial communities and may create environmental conditions that favour microorganisms capable of producing extracellular polymers involved in adhesion and particle aggregation. Consequently, pond water and sediment may serve as reservoirs for microorganisms with potential applications in biological water treatment [8].

The application of bioflocculant remains constrained by variations in production yield, flocculating efficiency, culture requirements, downstream recovery and performance under different water-treatment conditions. Therefore, the discovery of locally adapted microorganisms with strong bioflocculant-producing properties is an important step towards developing economical and environmentally compatible biological flocculants. The study aims to isolate bioflocculant producing microorganisms from fish ponds.

MATERIALS AND METHODS

Study Area

The study was conducted using samples collected from selected fish ponds located in Minna. Minna lies between latitudes 9°31' N and 9°45' N and longitudes 6°31' E and 6°39' E.

Sample Collection

Both pond water and sediment samples were collected aseptically from four selected fish ponds. Pond water samples were collected approximately 10–20 cm below the water surface using sterile, labelled sampling bottles. Sediment samples were collected from the pond bottom using sterile spatulas or appropriate sterile sediment-sampling equipment. Approximately 5–10 cm of the surface sediment was collected and transferred into sterile, labelled containers. Separate sampling materials were used for each sampling location to minimise cross-contamination [8]. The collected pond water and sediment samples were placed in an ice-packed carrier and transported to the Microbiology Laboratory of Federal university of Technology, Minna for analysis. Microbiological processing was commenced as soon as possible following sample collection.

Preparation of Pond Water Samples

The pond water samples were thoroughly mixed before serial dilution. One millilitre (1 mL) of each water sample was aseptically transferred into 9 mL of sterile normal saline to obtain the 10^{-1} dilution. Further ten-fold serial dilutions were prepared by transferring 1 mL from the preceding dilution into 9 mL of sterile normal saline, 10^{-3} dilutions were used for the isolation of bacterial and fungal microorganisms.

Preparation of Pond Sediment Samples

For each sediment sample, 1 g was aseptically transferred into 9 mL of sterile normal saline and thoroughly homogenised to obtain the initial 10^{-1} dilution. Further ten-fold serial dilutions were prepared by transferring 1 mL of the previous dilution into 9 mL of sterile

normal saline. 10^{-3} dilutions were selected for microbial isolation.

Enumeration of Microbial Isolates

Enumeration of microbial isolates both Bacteria and fungi was carried out to determine the abundance of bacteria and fungi in the pond water and sediment samples. Serial dilution and spread plate techniques were used, and plates were counted. Results were expressed as colony-forming units per millilitre (CFU/mL) for water and per gram (CFU/g) for sediment.

Isolation of Biofloculant-Producing Bacteria

Potential biofloculant-producing bacteria from both pond water and sediment samples were isolated using nutrient agar. Following preparation and sterilisation of the medium, plates were allowed to cool and solidify before use. Subsequently, one (1) mL of 3 dilution factor from each pond water and sediment sample was inoculated onto the prepared nutrient agar plates and spread evenly using a sterile spreader. The inoculated plates were incubated at $37\text{ }^{\circ}\text{C}$ for 24 h. Distinct colonies exhibiting differences in size, shape, colour, elevation, margin and surface characteristics were selected and sub-cultured to obtain pure isolates for further examination and subsequent screening for biofloculant-producing potential [9].

Isolation of Fungi

Fungi were isolated separately from the pond water and sediment samples using an appropriate fungal growth medium, such as Sabouraud Dextrose Agar (SDA). Appropriate dilutions of the water and sediment samples were inoculated onto the prepared medium and incubated at $28 \pm 2\text{ }^{\circ}\text{C}$ for approximately 5 days.

Distinct fungal colonies were selected based on colony colour, texture, pigmentation, growth pattern and other observable cultural characteristics. Selected colonies were subcultured onto fresh SDA plates to obtain pure fungal cultures. The purified isolates were maintained on SDA slants for subsequent screening and identification [10].

Purification and Preservation of Microbial Isolates

Distinct bacterial colonies recovered from both water and sediment samples were purified by repeated streaking onto Nutrient Agar (NA), while fungal isolates were purified on SDA. The bacterial plates were incubated at $37\text{ }^{\circ}\text{C}$ for 24–48 h and fungal plates at $28 \pm 2\text{ }^{\circ}\text{C}$ for 5 days. Pure cultures were identified by the presence of uniform colony morphology and absence of visible contamination. Each purified isolate was assigned a unique code indicating its sample source.

RESULTS

Microbial Colony Count of samples

Bacterial Colony Counts

The results shown in Table 1 revealed that bacterial colony counts were higher in sediment samples than in corresponding pond water samples across all four ponds. Sediment bacterial counts ranged from 1.26×10^5 to 1.75×10^5 CFU/mL, whereas water samples recorded counts between 1.10×10^4 and 9.4×10^4 CFU/mL. Among the sediment samples, Pond 4 recorded the highest bacterial count at 1.75×10^5 CFU/mL, followed by Pond 3 (1.58×10^5 CFU/mL), Pond 2 (1.43×10^5 CFU/mL) and Pond 1 (1.26×10^5 CFU/mL). This indicates that the sediment samples from Pond 4 yielded the greatest number of culturable bacteria among the sediment samples examined. Pond water samples

shows that Pond 3 recorded the highest bacterial count at 9.4×10^4 CFU/mL, while Pond 4 recorded the lowest count at 1.10×10^4 CFU/mL. The considerable

difference between water and sediment counts in Pond 4 suggests that the culturable bacterial population was more concentrated in the sediment sample

Table 1: Colony Count of bacterial Isolates from Pond Samples

Pond	Sample	Bacterial Count
P1	Water	8.5×10^4 CFU/mL
P1	Sediment	1.26×10^5 CFU/g
P2	Water	7.2×10^4 CFU/mL
P2	Sediment	1.43×10^5 CFU/g
P3	Water	9.4×10^4 CFU/mL
P3	Sediment	1.58×10^5 CFU/g
P4	Water	1.10×10^4 CFU/mL
P4	Sediment	1.75×10^5 CFU/g

Key: P-Pond

Fungal Colony Counts

The results show that fungal colony counts were consistently higher in sediment than in pond water across all four ponds. Sediment fungal counts ranged from 3.2×10^3 to 4.6×10^3 CFU/mL, while water counts ranged

from 1.8×10^3 to 2.7×10^3 CFU/mL. Pond 3 recorded the highest fungal count in both water (2.7×10^3 CFU/mL) and sediment (4.6×10^3 CFU/mL). Pond 1 recorded the lowest fungal counts in both water and sediment, with values of 1.8×10^3 and 3.2×10^3 CFU/mL, respectively.

Table 2: Colony Count of Bacterial Isolates from Pond Samples

Pond	Sample	Fungal Count
P1	Water	1.8×10^3 CFU/mL
P1	Sediment	3.2×10^3 CFU/g
P2	Water	2.2×10^3 CFU/mL
P2	Sediment	4.1×10^3 CFU/g
P3	Water	2.7×10^3 CFU/mL
P3	Sediment	4.6×10^3 CFU/g
P4	Water	2.5×10^3 CFU/mL
P4	Sediment	3.9×10^3 CFU/g

Key: P-Pond

Morphological Characteristics of Bacterial Isolates

The colony morphology of selected bacterial isolates obtained from the four (4) Ponds are presented in Table 2, Table 3, Table 4, Table 5, Table 6. The bacterial isolates displayed differences in colony shape, elevation, margin, surface texture and colour indication a rich bacterial community from both water and sediment. Circular colonies were observed among several isolates, while irregular colonies were also present. The colony elevations included raised, flat and convex forms, indicating morphological variation among the

bacterial isolates recovered from the pond. Surface characteristics included smooth, rough and mucoid textures. Isolates exhibited mucoid texture (P1W4, P1S4, P2W2, P2S4, P3W4, P3S4, P4W2, P4S4). Mucoid colonies are often associated with capsular polysaccharide production, which is a strong indicator of potential biofloculant activity. These isolates are particularly promising for further screening. Sediment samples generally yielded isolates with rougher, drier, and more irregular colony morphologies (P1S2, P1S5, P2S2, P2S5), while water isolates tended to be smoother and more mucoid. This may reflect adaptation to different environmental niches.

Table 3. Bacterial morphological characteristics of Isolates from Pond One (P1)

Isolate	Colony shape	Elevation	Margin	Surface/texture	Colour
P1W1	Circular	Raised	Entire	Smooth	Cream
P1W2	Irregular	Flat	Undulate	Rough	White
P1W3	Circular	Convex	Entire	Smooth	Yellow
P1W4	Circular	Raised	Entire	Mucoid	Cream
P1S1	Circular	Convex	Entire	Dry	White
P1S2	Irregular	Flat	Lobate	Rough	Cream
P1S3	Circular	Raised	Entire	Smooth	Pale yellow
P1S4	Circular	Convex	Entire	Mucoid	White
P1S5	Irregular	Flat	Undulate	Rough	Cream

Key: W- Water; S- Sediment; +- positive; - -Negative

Table 4. Bacterial morphological characteristics of Isolates from Pond Two (P2)

Isolate	Colony shape	Elevation	Margin	Surface/texture	Colour
P2W1	Circular	Raised	Entire	Smooth	Cream
P2W2	Circular	Convex	Entire	Mucoid	White
P2W3	Irregular	Flat	Undulate	Rough	Cream
P2W4	Circular	Raised	Entire	Smooth	Yellowish
P2S1	Circular	Convex	Entire	Dry	White
P2S2	Irregular	Flat	Lobate	Rough	Cream
P2S3	Circular	Raised	Entire	Smooth	Pale yellow
P2S4	Circular	Convex	Entire	Mucoid	Cream

Isolate	Colony shape	Elevation	Margin	Surface/texture	Colour
P2S5	Irregular	Flat	Undulate	Rough	White

Key: W- Water; S- Sediment; +- positive; - -Negative

Table 5. Bacterial morphological characteristics of Isolates from Pond Three (P3)

Isolate	Colony shape	Elevation	Margin	Surface/texture	Colour
P3W1	Circular	Raised	Entire	Smooth	Cream
P3W2	Irregular	Flat	Undulate	Rough	White
P3W3	Circular	Convex	Entire	Smooth	Yellow
P3W4	Circular	Raised	Entire	Mucoid	Cream
P3S1	Circular	Convex	Entire	Dry	White
P3S2	Irregular	Flat	Lobate	Rough	Cream
P3S3	Circular	Raised	Entire	Smooth	Pale yellow
P3S4	Circular	Convex	Entire	Mucoid	White
P3S5	Irregular	Flat	Undulate	Rough	Cream

Key: W- Water; S- Sediment; +- positive; - -Negative

Table 6. Bacterial morphological characteristics of Isolates from Pond Four (P4)

Isolate	Colony shape	Elevation	Margin	Surface/texture	Colour
P4W1	Circular	Raised	Entire	Smooth	Cream
P4W2	Circular	Convex	Entire	Mucoid	White
P4W3	Irregular	Flat	Undulate	Rough	Cream
P4W4	Circular	Raised	Entire	Smooth	Yellow
P4S1	Circular	Convex	Entire	Dry	White

Isolate	Colony shape	Elevation	Margin	Surface/texture	Colour
P4S2	Irregular	Flat	Lobate	Rough	Cream
P4S3	Circular	Raised	Entire	Smooth	Pale yellow
P4S4	Circular	Convex	Entire	Mucoid	Cream
P4S5	Irregular	Flat	Undulate	Rough	White

Key: W- Water; S- Sediment; +- positive; - -Negative

Biochemical characteristics of selected bacterial isolates

The biochemical characterization of selected bacterial isolates across Tables 7, Table 8, Table 9, Table 10 comprising of the four ponds provided valuable insights into the identity and metabolic capabilities of the bacterial community. A total of twenty isolates were identified across the four ponds, with five isolates from each pond. The most frequently isolated bacteria were *Bacillus* species, accounting for six out of the twenty isolates, followed by *Pseudomonas* species with three isolates. Other genera identified included *Staphylococcus*, *Escherichia*, *Enterobacter*, *Micrococcus*, *Aeromonas*, *Klebsiella*, *Acinetobacter*, *Proteus*, and *Citrobacter*. The

biochemical profiles showed that almost all isolates were catalase-positive, with only P1SB4 identified as *Escherichia coli* and P4B4 identified as *Citrobacter* species being catalase-negative. Oxidase activity was positive for *Pseudomonas*, *Aeromonas*, and *Acinetobacter* species, while indole production was positive for *Escherichia coli*, *Enterobacter*, *Proteus* and *Citrobacter* species. Citrate utilization was variable with many isolates testing positive, urease activity was positive for *Staphylococcus*, *Bacillus* species, *Klebsiella*, *Proteus* and *Citrobacter*, and motility was observed in most isolates except some *Staphylococcus* species and *Bacillus* species. Both sediment and water niches harboured diverse potential bioflocculant producers.

Table 7. Biochemical characteristics of selected bacterial isolates from Pond 1

Isolate	Gram reaction	Catalase	Oxidase	Indole	Citrate	Urease	Motility	Suspected identity
P1WB1	+	+	—	—	+	—	+	<i>Bacillus</i> sp.
P1WB2	—	+	+	—	+	—	+	<i>Pseudomonas</i> sp.
P1SB3	+	+	—	—	—	+	—	<i>Staphylococcus</i> sp.
P1SB4	—	—	—	+	—	—	+	<i>Escherichia coli</i>

Isolate	Gram reaction	Catalase	Oxidase	Indole	Citrate	Urease	Motility	Suspected identity
P1SB5	+	+	–	–	+	+	+	<i>Bacillus</i> sp.

Table 8. Biochemical characteristics of selected bacterial isolates from Pond 2

Isolate	Gram reaction	Catalase	Oxidase	Indole	Citrate	Urease	Motility	Suspected identity
P2WB1	–	+	–	+	+	–	+	<i>Enterobacter</i> sp.
P2WB2	+	+	–	–	+	–	+	<i>Micrococcus</i> sp.
P2SB3	–	+	+	–	+	–	+	<i>Aeromonas</i> sp.
P2SB4	+	+	–	–	+	+	+	<i>Bacillus</i> sp.
P2SB5	–	+	–	–	+	+	+	<i>Klebsiella</i> sp.

Table 9. Biochemical characteristics of selected bacterial isolates from Pond 3

Isolate	Gram reaction	Catalase	Oxidase	Indole	Citrate	Urease	Motility	Suspected identity
P3WB1	–	+	+	–	+	–	+	<i>Acinetobacter</i> sp.
P3WB2	+	+	–	–	+	–	+	<i>Bacillus</i> sp.
P3SB3	–	+	+	–	–	–	+	<i>Pseudomonas</i> sp.
P3SB4	+	+	–	–	–	+	–	<i>Staphylococcus</i> sp.
P3SB5	–	+	–	+	+	+	+	<i>Proteus</i> sp.

Table 10. Biochemical characteristics of selected bacterial isolates from Pond 4

Isolate	Gram reaction	Catalase	Oxidase	Indole	Citrate	Urease	Motility	Suspected identity
P4B1	+	+	–	–	+	–	+	<i>Bacillus</i> sp.
P4B2	+	+	–	–	–	–	–	<i>Staphylococcus</i> sp.
P4B3	–	+	+	–	+	–	+	<i>Pseudomonas</i> sp.
P4B4	–	–	–	+	–	+	+	<i>Citrobacter</i> sp.
P4B5	+	+	–	–	+	+	+	<i>Bacillus</i> sp.

Morphological Characteristics of Fungal Isolates

The morphological characterization of fungal isolates across Tables 11 to Table 14 revealed a diverse range of fungal genera in the pond ecosystems. A total of twelve fungal isolates were examined, with three isolates from each pond. The fungi isolated included *Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium*, *Rhodotorula*, *Trichoderma*, *Mucor*, *Cladosporium* and yeasts. Several of these genera are well-known biofloculant producers, including *Aspergillus* species such as *Aspergillus niger* and *Aspergillus flavus* which were isolated from P1, P3, and P4, *Penicillium* species which were isolated from P1, *Rhizopus* species which were isolated from P2, *Fusarium* species were isolated from P2, *Trichoderma* species which

were isolated from P3, and *Mucor* species which were isolated from P4. *Cladosporium* species isolated from P4. Yeasts including *Rhodotorula* species and other yeast species were isolated from P1, P2, and P3. The distribution of fungi across ponds showed that P1 yielded *Aspergillus*, *Penicillium*, and yeast, P2 yielded *Rhizopus*, *Fusarium*, and *Rhodotorula*, P3 yielded *Aspergillus*, *Trichoderma*, and yeast, while P4 yielded *Aspergillus*, *Mucor*, and *Cladosporium*. Both water and sediment yielded fungal isolates, with water isolates including *Aspergillus* from P1, P3, and P4, and *Rhizopus* from P2, while sediment isolates included *Penicillium*, yeast, *Fusarium*, *Rhodotorula*, *Trichoderma*, *Mucor*, and *Cladosporium*, indicating that sediment appears to harbour a wider diversity of fungi.

Ta

ble 11. Morphological characteristics of fungal isolates — P1

Isolate	Colony colour	Texture	Microscopic characteristics	Suspected Organism
P1WF1	White	Cottony	Septate hyphae, conidial structures	<i>Aspergillus</i> sp.
P1SF2	Greenish	Powdery	Septate hyphae and conidia	<i>Penicillium</i> sp.
P1SF3	Cream	Smooth	Oval yeast-like cells	Yeast sp.

Table 12. Morphological characteristics of fungal isolates — P2

Isolate	Colony colour	Texture	Growth	Microscopic characteristics	Suspected Organism
P2WF1	White brownish	Cottony	Rapid	Broad, aseptate hyphae with sporangial	Rhizopus sp.
P2SF2	yellowish	velvety	Moderate	Septate hyphae macroconidia	Fusarium sp.
P2SF3	Cream	Mucoid	Moderate	Oval, elongated	Rhodotorula sp.

Table 13. Morphological characteristics of fungal isolates — P3

Isolate	Colony colour	Texture	Growth	Microscopic characteristics	Suspected Organism
P3WF1	Blackish green	Powdery	Rapid	Septate hyphae and conidia	<i>Aspergillus</i> sp.
P3SF2	grey	cottony	rapid	Septate hyphae with brush like conidiophores	<i>Trichoderma</i> sp.
P3SF3	Cream	Smooth	Moderate	Oval budding cells	Yeast sp.

Table 14. Morphological characteristics of fungal isolates — P4

Isolate	Colony colour	Texture	Growth	Microscopic characteristics	Suspected Organism
P4WF1	White	Cottony	Rapid	Septate hyphae and conidia	<i>Aspergillus</i> sp.
P4SF2	White grey	Cottony, fluffy	Moderate	Septate hyphae	<i>mucor</i> sp.
P4SF3	yellowish	velvety	Moderate	Septate hyphae, conidia in cluster	<i>Cladosporium</i> sp.

DISCUSSION

The present study was designed to isolate and characterize biofloculant-producing microorganisms from fish pond water and sediment, based on the premise that aquatic environments, particularly aquaculture systems, harbour diverse microbial communities with the capacity to produce extracellular polymeric substances capable of flocculating suspended particles [8,9]. The results obtained provide compelling evidence that both water and sediment from the sampled fish ponds contain a rich array of bacteria and fungi with significant biofloculant-producing potential.

The bacterial colony counts obtained in this study revealed that sediment samples from all four ponds yielded substantially higher bacterial loads than corresponding water samples. Sediment counts ranged from 1.26×10^5 to 1.75×10^5 CFU/mL, while water counts ranged from 1.10×10^4 to 9.4×10^4 CFU/mL. The observation agrees with the report of Mallik *et al.* [11] that ecological principles favour sediment, as sediment provides a nutrient-rich, physically stable, and protected microenvironment that favours microbial colonization, biofilm formation, and metabolic activity. The sediment interface is known to concentrate organic matter, nitrogenous compounds, and minerals, creating a favourable niche for diverse microbial populations [12]. This finding is significant because it suggests that sediment should be the primary target for isolation of diverse microorganisms for biofloculant potential. However, it is important to acknowledge that high colony counts do not necessarily correlate with high biofloculant production, as the trait is species-specific and often strain-specific.

The fungal colony counts, while lower than bacterial counts, followed a similar sediment-water distribution pattern. Sediment fungal counts ranged from 3.2×10^3 to 4.6×10^3 CFU/mL, while water counts ranged from 1.8×10^3 to 2.7×10^3 CFU/mL. The lower fungal abundance relative to bacteria is consistent with the general ecological observation that bacteria dominate aquatic microbial communities, particularly in nutrient-rich environments [13,14]. Fungal counts peaked in P3 sediment (4.6×10^3 CFU/mL) suggests that this pond may harbour a particularly favourable environment for fungal growth, possibly due to specific nutrient profiles or organic matter composition [15]. However, as with bacteria, the presence of fungi does not guarantee biofloculant production, and the relatively lower abundance of fungi compared to bacteria may mean that bacterial isolates will ultimately prove more productive in screening programmes. Nonetheless, the isolation of fungi from all ponds justifies their inclusion in biofloculant screening efforts, particularly given the well-documented flocculating activity of genera such as *Aspergillus*, *Penicillium*, and *Rhizopus*.

The morphological characterization of bacterial isolates revealed considerable diversity in colony shape, elevation, margin, surface texture, and colour, indicating a taxonomically and metabolically diverse bacterial community. The predominance of circular colony shapes with raised or convex elevations and entire margins among many isolates is typical of many common aquatic bacteria, including *Bacillus* and *Pseudomonas* species [16]. Of particular interest for biofloculant screening is the observation that several isolates exhibited mucoid colony texture, specifically P1W4, P1S4, P2W2, P2S4, P3W4, P3S4, P4W2, and P4S4. Mucoid colonies are strongly associated with the

production of capsular polysaccharides and extracellular polymeric substances, which are the primary agents responsible for bioflocculation [17]. The presence of eight mucoid isolates across all four ponds is therefore highly encouraging and suggests that bioflocculant production may be a relatively common trait in these environments. However, it is critical to note that mucoid phenotype alone is not sufficient evidence of bioflocculant activity, as some capsular polysaccharides may lack flocculating properties, and some bioflocculant producers may not exhibit a mucoid phenotype under standard culture conditions. Therefore, while mucoid isolates should be prioritized for quantitative flocculation assays, non-mucoid isolates should not be excluded from screening, as they may produce bioflocculants that are secreted into the medium rather than retained as a capsule [18].

The biochemical characterization of selected bacterial isolates provided preliminary identification of twenty isolates across the four ponds, with *Bacillus* species being the most frequently isolated genus, followed by *Pseudomonas* species. This finding is highly significant because bioflocculant has been discovered to be produced by *Bacillus* and *Pseudomonas* and are among the most extensively studied genera for bioflocculant production [5, 19]. *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus cereus* and other *Bacillus* species have been reported to produce a variety of bioflocculants, including polysaccharides, proteins, and glycoproteins, with flocculating activities comparable to or exceeding those of synthetic flocculants [7, 20]. Similarly, *Pseudomonas* species such as *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Pseudomonas putida* are known bioflocculant producers, with

some strains producing high-molecular-weight exopolysaccharides with excellent flocculating capacity. The isolation of these genera from fish pond environments is not surprising, as they are ubiquitous in aquatic ecosystems and are well-adapted to the nutrient-rich conditions found in aquaculture systems [19].

The fungal isolates obtained in this study represented a diverse range of genera, including *Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium*, *Rhodotorula*, *Trichoderma*, *Mucor*, *Cladosporium*, and yeasts. Several of these genera are well-known bioflocculant producers, particularly *Aspergillus*, *Penicillium*, and *Rhizopus*, which have been extensively studied for their ability to produce polysaccharide-based bioflocculants [21]. The isolation of *Aspergillus* species from P1, P3, and P4 suggests that this genus is widespread in the pond environments and may play a significant role in natural flocculation processes. *Penicillium* species, isolated from P1, are also known bioflocculant producers, although their flocculating activity is generally lower than that of *Aspergillus* species. *Rhizopus* species, isolated from P2, are capable of producing chitosan-based bioflocculants, which have unique properties and potential applications [22]. *Fusarium* species, isolated from P2, have been reported to produce exopolysaccharides with flocculating activity, although some *Fusarium* species are plant pathogens and mycotoxin producers, which raises biosafety concerns [10]. *Trichoderma* species, isolated from P3, are known for their antagonistic activity against plant pathogens and their ability to produce polysaccharide-degrading enzymes, but some species also produce bioflocculants. *Mucor* species, isolated from P4, are known to produce chitosan and other polysaccharides with flocculating properties. *Cladosporium*

species, isolated from P4, are less commonly reported for biofloculant production, but some species produce extracellular polysaccharides that may exhibit flocculating activity [23].

The presence of yeasts, including *Rhodotorula* species and other yeast species, in P1, P2, and P3 is important because yeasts are increasingly recognized as potential biofloculant producers. *Rhodotorula* species are known to produce extracellular polysaccharides and lipids, and some strains have demonstrated flocculating activity. Yeasts have several advantages over filamentous fungi and bacteria for biofloculant production, including rapid growth, ease of cultivation, and the ability to utilize a wide range of carbon sources. However, the flocculating activity of yeast-derived biofloculants is often lower than that of bacterial or filamentous fungal biofloculants, and optimization of production conditions may be necessary to achieve commercially viable yields [24, 25].

The study provides a valuable foundation for further research on biofloculant production from fish pond microorganisms. The isolates identified in this study represent a diverse and promising collection of candidates for biofloculant production, and the findings support the report that aquaculture environments are rich sources of microorganisms with biotechnological potential.

CONCLUSION

The results of this study demonstrated that fish pond water and sediment harbour diverse communities of bacteria and fungi with significant potential for biofloculant production. The consistent dominance of sediment over water as a source of microbial isolates, the prevalence of known biofloculant-producing genera such as *Bacillus*,

Pseudomonas, *Aspergillus*, *Penicillium*, and *Rhizopus*, has the potential to produce capsular polysaccharide that support the feasibility of isolating effective biofloculant producers from these environments. However, the study also highlights the need for quantitative assays, molecular identification, biosafety assessment, and optimization studies to translate these findings into practical applications. With further research, the microorganisms isolated in this study could contribute to the development of sustainable biofloculants for water and wastewater treatment as well as other environmental applications.

Authors Contribution

Conceptualization: Bala Jeremiah David; Maude Asma'u Mohammed and Abioye Olabisi Peter; Supervision: Bala Jeremiah David and Oyewole Oluwafemi Adebayo; Investigation: Bala Jeremiah David and Musa John Jiya; Writing the first draft of the article: Job Oloruntoba Samuel; Editing: Bala Jeremiah David, Abioye Olabisi Peter and Oyewole Oluwafemi Adebayo; Maude Asma'u Mohammed. All authors contributed to the development of the final manuscript and approved its submission.

Disclosure of Conflict of Interest

None

Ethics Approval and Informed Consent

This study did not use human or animal subjects. Therefore, ethical consideration was not applicable.

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