

Biodegradation of Pollutants in *Manihot esculenta* (Cassava) Wastewater by *Bacillus* and *Pseudomonas* Species: An Assessment of Physicochemical and Microbial Dynamics

Oluwasanmi Anuoluwapo Adeyemi^{1*}, Busola Victoria Baoku¹, Jeremiah Ikhevha Ogah², Olaoluwa Temitope Talabi³, Oyindamola John Samson⁴

¹ Department of Microbiology and Biotechnology, Ajayi Crowther University, Oyo, Oyo State, Nigeria.

² Infectious Diseases and Environmental Health Research Group, University of Ilorin, Ilorin, Nigeria.

³ Department of Biochemistry, Faculty of Life Sciences, University of Lagos, Lagos, Nigeria.

⁴ Department of Microbiology, Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria.

*Correspondence: Oluwasanmi Anuoluwapo ADEYEMI: oa.adeyemi@acu.edu.ng

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Abstract

The discharge of untreated cassava processing wastewater poses environmental and public health challenges in tropical regions, particularly in Nigeria, the world's largest cassava producer, where bioremediation studies targeting this effluent using native isolates remain limited. This study investigated the biodegradative potential of *Bacillus cereus*, *Bacillus subtilis*, and two strains of *Pseudomonas aeruginosa* isolated from *Manihot esculenta* (cassava) wastewater collected from a garri processing facility in Oyo, Oyo State, Nigeria. Isolates were purified by successive subculturing and propagated to standardised inoculum concentrations before treating wastewater samples over 144 hours. Physicochemical parameters (pH, electrical conductivity, TS, TSS, TDS, DO, and BOD₅) and heavy metals (Pb, Cu, and Zn) were determined at 0, 24, 72, and 144 hours following APHA standard methods. Total cyanide was quantified spectrophotometrically using the picric acid method. Microbial community composition was assessed by plate counts on selective and differential media. One-way ANOVA with Duncan's Multiple Range Test ($p \leq 0.05$) was applied to detect treatment differences. All removal efficiencies were statistically significant ($p \leq 0.05$). *B. subtilis* achieved the highest cyanide removal (82.7%), while both *Bacillus* and *Pseudomonas* species significantly reduced heavy metals (Pb: 51–67%; Cu: 54–66%; Zn: 53–64%), BOD₅ (50–63%), and TSS (49–59%). pH increased significantly from 4.2 to 6.4 ($p \leq 0.05$), approaching neutral conditions suitable for discharge. Microbial community analysis revealed a shift from diverse initial flora to *Bacillus*-dominated communities post-treatment. These findings establish *Bacillus* and *Pseudomonas* species as effective, sustainable bioremediation agents for cassava wastewater management in developing nations.

Keywords: *Bacillus subtilis*, Bioremediation; Cassava wastewater; Cyanide; Heavy metal removal; Physicochemical parameters; *Pseudomonas aeruginosa*

1. Introduction

Cassava (*Manihot esculenta* Crantz) is an important tuber crop cultivated in subtropical and tropical regions. It serves as a fundamental source of food for over five hundred million people globally [1]. Nigeria is the world's largest cassava producer, responsible for approximately 20% of global output with over 63 million tonnes produced annually [2, 3]. Nigeria's cassava production has experienced consistent growth, rising from approximately 33 million tonnes in 1999 to over 60 million tonnes in recent years [7,

8]. The cassava processing industry, particularly garri production, generates substantial quantities of wastewater characterised by high organic load, cyanogenic compounds, heavy metals, and elevated biochemical oxygen demand [4, 5].

Cassava wastewater contains cyanogenic glycosides (linamarin and lotaustralin), which are toxic because they release hydrogen cyanide (HCN). HCN is a highly toxic compound with concentrations ranging from 86 to 274 mg/L in untreated effluents [5]. The indiscriminate discharge of these effluents into the environment

causes severe ecological damage, including soil acidification, groundwater contamination, eutrophication of receiving water bodies, and toxicity to aquatic organisms [6]. Conventional treatment methods for cassava wastewater, including alkaline chlorination, oxidation, and physical or chemical processes, are often energy-intensive, expensive, and may generate secondary pollutants [5, 24]. These limitations necessitate the exploration of sustainable biological alternatives.

Biological treatment approaches utilising indigenous microorganisms have emerged as sustainable, cost-effective alternatives [13, 25]. *Bacillus* and *Pseudomonas* species have demonstrated remarkable metabolic versatility in degrading various organic pollutants, including cyanide compounds, through enzymatic pathways involving cyanide hydratase, nitrilase, and rhodanese [9, 10]. Previous studies have verified the effectiveness of *Bacillus pumilus* and *Pseudomonas putida* in achieving up to 63% and 61% cyanide removal, respectively [11, 12]. Similarly, *Pseudomonas fluorescens* and *Bacillus pumilus* consortia have reduced cyanide levels by over 80% while simultaneously remediating heavy metals in contaminated soils [13]. The *Bacillus* genus comprises rod-shaped, Gram-positive bacteria belonging to the phylum Bacillota, characterised by their ability to form endospores that can survive extreme environmental conditions [16]. *Pseudomonas* is a genus of Gram-negative bacteria belonging to the Pseudomonadaceae family, exhibiting remarkable metabolic diversity that enables survival in diverse environments [17]. Furthermore, *B. subtilis* has been successfully utilised for biosurfactant production using cassava wastewater as substrate, demonstrating its dual potential for waste valorisation and treatment [14, 15].

Despite these advances, comprehensive studies evaluating the comparative biodegradative efficiency of multiple *Bacillus* and *Pseudomonas* species on cassava wastewater under controlled conditions remain limited, particularly in the Nigerian context. This study therefore aimed to: (1) isolate and characterise *Bacillus* and *Pseudomonas* species from cassava wastewater; (2) evaluate their biodegradative potential through comprehensive physicochemical analysis; (3) compare the treatment efficiency of different bacterial species; and (4) elucidate the microbial

community dynamics during the degradation process.

2. Materials and Methods

2.1 Study Area and Sample Collection

Cassava wastewater samples were collected from a commercial garri processing facility located in Oyo Alaafin Town, Oyo State, Nigeria (lat. 7°51'00"N; long. 3°55'60"E) [20] in March 2024. The facility processes fresh cassava tubers through peeling, washing, grating, fermentation, and dewatering stages, generating wastewater primarily from the pressing of fermented cassava mash. Fifteen samples (5 L each) were collected in sterile high-density polyethylene containers during morning hours (07:00–09:00 h) when processing activities were at peak, and transported to the laboratory within 2 hours under refrigerated conditions (4°C) for analysis [20].

2.2 Isolation and Identification of Bacterial Isolates

Bacterial isolation was performed using standard microbiological techniques [21]. Serial dilutions (10^{-1} to 10^{-6}) of wastewater samples were prepared in sterile physiological saline (0.85% NaCl). Aliquots (1 mL) from dilutions 10^{-5} and 10^{-6} were inoculated onto Nutrient Agar (Oxoid, UK) plates using the pour plate technique and incubated aerobically at 37°C for 24–48 hours. Morphologically discrete colonies were subcultured to obtain pure isolates.

Bacterial identification was performed through morphological characterisation (colony morphology, Gram staining, endospore staining) and biochemical testing, including catalase, oxidase, starch hydrolysis, citrate utilisation, sugar fermentation (glucose, lactose, mannitol), Voges–Proskauer, and methyl red tests [21]. Isolates were identified using Bergey's Manual of Determinative Bacteriology.

2.3 Experimental Design for Biodegradation Studies

Four bacterial treatments were evaluated: *Bacillus cereus* (BC), *Bacillus subtilis* (BS), *Pseudomonas aeruginosa* strain 1 (PA1), and *P. aeruginosa* strain 2 (PA2). All isolates were obtained from the cassava wastewater samples collected as described in Section 2.1. Bacterial inocula were prepared by cultivating pure isolates in Nutrient Broth (50 mL) at 37°C for 24 hours with shaking (150 rpm). The resulting cultures ($\sim 10^8$ CFU/mL,

confirmed by viable plate count) were added to 500 mL cassava wastewater samples in sterile 1 L beakers. An uninoculated wastewater sample served as the negative control. All treatments were conducted in triplicate ($n = 3$ per group) and incubated at ambient temperature (28–32°C) for 144 hours. Aliquots (50 mL) were withdrawn at 0, 24, 72, and 144 hours for bacteriological and physicochemical analyses.

2.4 Physicochemical Analysis

Standard analytical methods were used to characterise sample physicochemical properties [20]. Water temperature was measured with a calibrated mercury thermometer. pH was determined using a Hanna Instruments digital meter (HI9813-6), calibrated against buffer solutions at pH 4.0, 7.0, and 9.0. Electrical conductivity was measured with a Hanna Instruments conductivity meter (HI8733). Total solids (TS), total suspended solids (TSS), and total dissolved solids (TDS) were each determined gravimetrically by weighing dried residues according to APHA Method 2540 [20]. Dissolved oxygen (DO) was recorded with a YSI Pro20 dissolved oxygen meter (YSI Incorporated, Yellow Springs, OH, USA). Five-day biochemical oxygen demand (BOD₅) was determined by incubating samples in the dark at 20°C for five days and computing the difference between initial and final DO readings (APHA Method 5210B) [20].

2.5 Cyanide and Heavy Metal Analysis

Total cyanide concentration was determined spectrophotometrically using the picric acid method (APHA Method 4500-CN⁻ F) [20]. Briefly, samples were distilled to isolate cyanide as HCN, which was then absorbed in sodium hydroxide and reacted with picric acid; absorbance was read at 520 nm against a standard calibration curve. Heavy metals (copper, lead, and zinc) were analysed by Atomic Absorption Spectrophotometry (AAS) (Perkin Elmer AAnalyst 400, Waltham, MA, USA) following acid digestion with concentrated HNO₃ and HCl (3:1 v/v) per USEPA Method 3050B [23].

2.6 Statistical Analysis and Comparative Treatment Efficiency

All measurements were assayed in triplicate, and results were reported as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) to assess differences across treatments at each timepoint. Duncan's Multiple Range Test (DMRT) was applied post-hoc to identify significantly differing group pairs at $p \leq 0.05$. Pollutant removal efficiency was calculated as: Removal (%) = $[(C_i - C_f) / C_i] \times 100$, where C_i = initial concentration and C_f = final concentration at 144 hours. Comparative treatment efficiency across all parameters was compiled from the results of sections 3.2–3.4 and summarised graphically (Figure 6) to identify the most effective treatment for each pollutant class.

3. Results

3.1 Bacterial Enumeration and Community Dynamics

The initial bacterial count in untreated cassava wastewater was 1.12×10^7 CFU/mL (Table 1; Figure 1). Following inoculation, all treated samples exhibited a significant ($p \leq 0.05$) characteristic growth pattern: a marked increase at 24 hours, followed by a gradual decline through 72 and 144 hours. *Bacillus cereus* (BC) achieved the highest peak count (6.0×10^7 CFU/mL) at 24 hours, while *P. aeruginosa* strain 2 (PA2) exhibited the lowest (4.8×10^7 CFU/mL). By 144 hours, bacterial counts had declined to $1.5\text{--}2.5 \times 10^7$ CFU/mL across all treated samples — significantly higher than the untreated control (1.08×10^7 CFU/mL; $p \leq 0.05$) — indicating substrate depletion and transition to the stationary/death phase (Figure 1; Table 1). Differences among all treatment groups at each timepoint were statistically significant ($F_{4,10} = 22.6\text{--}38.4$, $p \leq 0.001$).

Table 1. Bacterial population counts (CFU/mL $\times 10^7$) across treatments at each sampling interval

Treatment	0 h	24 h	72 h	144 h
Control (untreated)	1.12 $\pm$ 0.04	1.15 $\pm$ 0.03 ^d	1.10 $\pm$ 0.05 ^d	1.08 $\pm$ 0.04 ^d
BC (<i>B. cereus</i>)	1.12 $\pm$ 0.04	6.0 $\pm$ 0.21 ^a	3.8 $\pm$ 0.18 ^a	2.5 $\pm$ 0.14 ^a
BS (<i>B. subtilis</i>)	1.12 $\pm$ 0.04	5.6 $\pm$ 0.19 ^b	3.5 $\pm$ 0.16 ^b	2.3 $\pm$ 0.11 ^b
PA1 (<i>P. aeruginosa</i> 1)	1.12 $\pm$ 0.04	5.1 $\pm$ 0.17 ^c	3.2 $\pm$ 0.14 ^c	1.9 $\pm$ 0.10 ^c
PA2 (<i>P. aeruginosa</i> 2)	1.12 $\pm$ 0.04	4.8 $\pm$ 0.15 ^c	3.0 $\pm$ 0.13 ^c	1.5 $\pm$ 0.09 ^d

Values are presented as mean $\pm$ SD (n = 3). Values in a column sharing the same superscript letter do not differ significantly ($p > 0.05$, DMRT). BC: *B. cereus*; BS: *B. subtilis*; PA1/PA2: *P. aeruginosa* strains. Different superscript letters in a column indicate statistically significant difference ($p \leq 0.05$, DMRT).

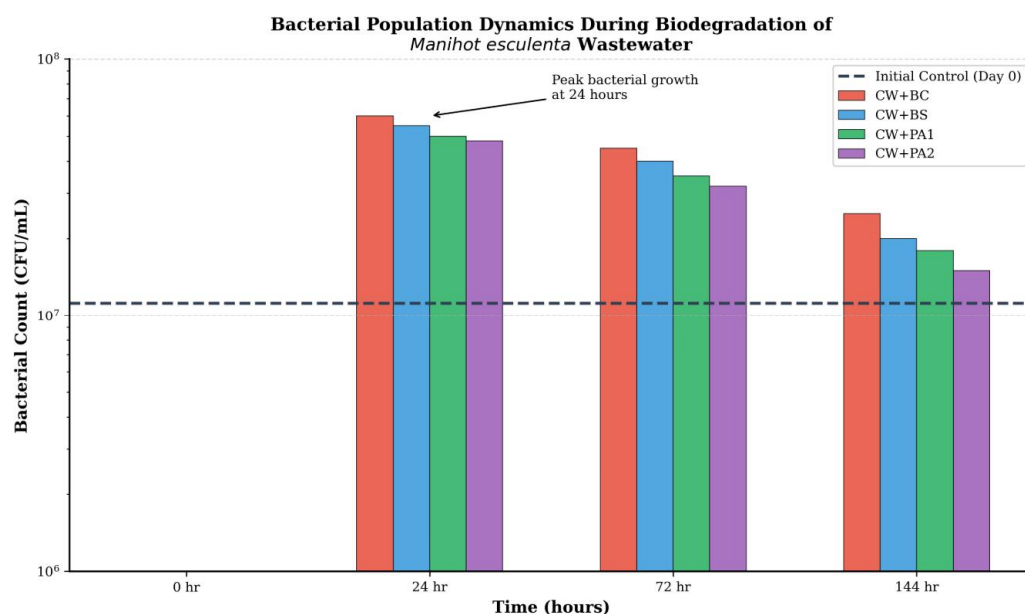


Figure 1. Bacterial population dynamics during biodegradation of *Manihot esculenta* (cassava) wastewater over 144 hours.

BC: *B. cereus*; BS: *B. subtilis*; PA1/PA2: *P. aeruginosa* strains. Bars represent mean $\pm$ SD (n = 3).

Microbial community analysis revealed a significant shift in bacterial composition during treatment ($p \leq 0.05$; Figure 2). The initial wastewater harboured a diverse community comprising *Bacillus* spp. (35%), *Pseudomonas* spp. (25%), *Micrococcus* spp. (15%),

Staphylococcus spp. (15%), and *Streptococcus* spp. (10%). By 144 hours, community diversity declined significantly, with *Bacillus* spp. (55%) and *Pseudomonas* spp. (35%) dominating, while other genera decreased to a combined 10%.

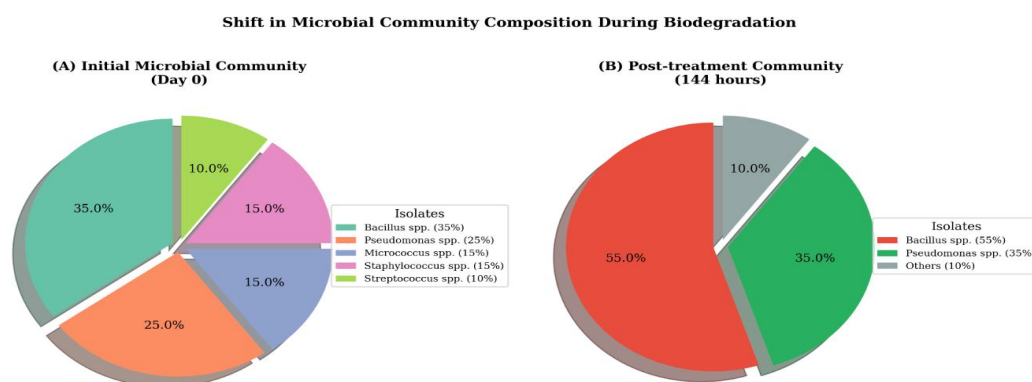


Figure 2. Shift in microbial community composition during biodegradation.

(A) Initial microbial community at Day 0; (B) post-treatment community at 144 hours. Values shown are percentage contributions of each genus. *Bacillus* spp. increased from 35% to 55%; *Pseudomonas* spp. from 25% to 35%; other genera declined collectively from 40% to 10%.

3.2 Changes in Physicochemical Parameters

Significant changes in all physicochemical parameters were observed across treatments ($F_{4,10} = 8.4\text{--}44.6$, $p \leq 0.05$; Table 2; Figure 3). Temperature increased progressively from 28.5°C in the untreated control to 30.4–31.5°C at 144 hours, with BC treatment recording the highest increase (31.5 °C; Table 2). Temperature values in treated samples differed significantly from the control at 144 hours ($p \leq 0.05$). Electrical conductivity (EC) increased significantly from 2850 $\mu\text{S}/\text{cm}$ to 3400–3580 $\mu\text{S}/\text{cm}$ across all treated samples by 144 hours ($p \leq 0.05$; Figure 3C; Table 2), reflecting increased ion release during organic matter decomposition.

The pH demonstrated a notable increase from the initial acidic value of 4.2 to 5.8–6.4 at 144 hours,

with BS treatment achieving the highest final pH of 6.4 ($p \leq 0.05$; Figure 3B). Total solids (TS) decreased significantly from 12,500 mg/L to 7,800–8,800 mg/L (30–38% removal; $p \leq 0.05$), with BS achieving the highest reduction (38%; Figure 3D; Table 2). Total suspended solids (TSS) exhibited substantial reduction from 4,800 mg/L to 1,950–2,450 mg/L (49–59% removal; $p \leq 0.05$), while total dissolved solids (TDS) increased from 7,700 mg/L to 8,100–8,650 mg/L, reflecting solubilisation of particulate matter during bacterial metabolism. Dissolved oxygen (DO) decreased significantly from 3.2 mg/L to 1.6–2.1 mg/L ($p \leq 0.05$; Table 2), indicating active aerobic respiration. BOD₅ was reduced significantly from 4,500 mg/L to 1,650–2,250 mg/L (50–63% removal; $p \leq 0.05$; Figure 3E).

Table 2. Physicochemical parameters of cassava wastewater before and after 144 h bacterial treatment

Parameter	Control	BC	BS	PA1	PA2	FEPA Limit
pH	4.2 $\pm$ 0.1 ^c	6.1 $\pm$ 0.1 ^b	6.4 $\pm$ 0.1 ^a	5.9 $\pm$ 0.1 ^c	5.8 $\pm$ 0.1 ^d	6.0–9.0
Temp. (°C)	28.5 $\pm$ 0.2 ^d	31.5 $\pm$ 0.3 ^a	30.8 $\pm$ 0.2 ^b	31.0 $\pm$ 0.3 ^{ab}	30.4 $\pm$ 0.2 ^c	≤ 40
EC ($\mu\text{S}/\text{cm}$)	2850 $\pm$ 42 ^c	3540 $\pm$ 38 ^a	3480 $\pm$ 31 ^b	3400 $\pm$ 29 ^c	3410 $\pm$ 35 ^c	≤ 1000
TS (mg/L)	12500 $\pm$ 124 ^a	8800 $\pm$ 98 ^c	7800 $\pm$ 87 ^d	8500 $\pm$ 94 ^c	8650 $\pm$ 91 ^b	≤ 10000
TSS (mg/L)	4800 $\pm$ 62 ^a	2300 $\pm$ 41 ^c	1950 $\pm$ 35 ^d	2250 $\pm$ 38 ^c	2450 $\pm$ 44 ^b	≤ 30
TDS (mg/L)	7700 $\pm$ 91 ^d	8500 $\pm$ 72 ^b	8650 $\pm$ 68 ^a	8100 $\pm$ 63 ^c	8200 $\pm$ 67 ^c	≤ 2000
DO (mg/L)	3.2 $\pm$ 0.1 ^a	1.8 $\pm$ 0.1 ^c	2.1 $\pm$ 0.1 ^b	1.6 $\pm$ 0.1 ^d	1.7 $\pm$ 0.1 ^d	≥ 5.0
BOD ₅ (mg/L)	4500 $\pm$ 87 ^a	2100 $\pm$ 48 ^c	1650 $\pm$ 39 ^d	2000 $\pm$ 43 ^c	2250 $\pm$ 52 ^b	≤ 50

Values are presented as mean $\pm$ SD, ($n = 3$). Values in a row sharing the same superscript letter do not differ significantly ($p > 0.05$, DMRT).

BC: *B. cereus*; BS: *B. subtilis*; PA1/PA2: *P. aeruginosa* strains. EC: electrical conductivity; TS: total solids; TSS: total suspended solids; TDS: total dissolved solids; DO: dissolved oxygen; BOD₅: five-day biochemical oxygen demand. FEPA: Federal Environmental Protection Agency, Nigeria. Different superscript letters in a row indicate statistically significant difference ($p \leq 0.05$, DMRT).

Physicochemical Parameters of Cassava Wastewater During Biodegradation

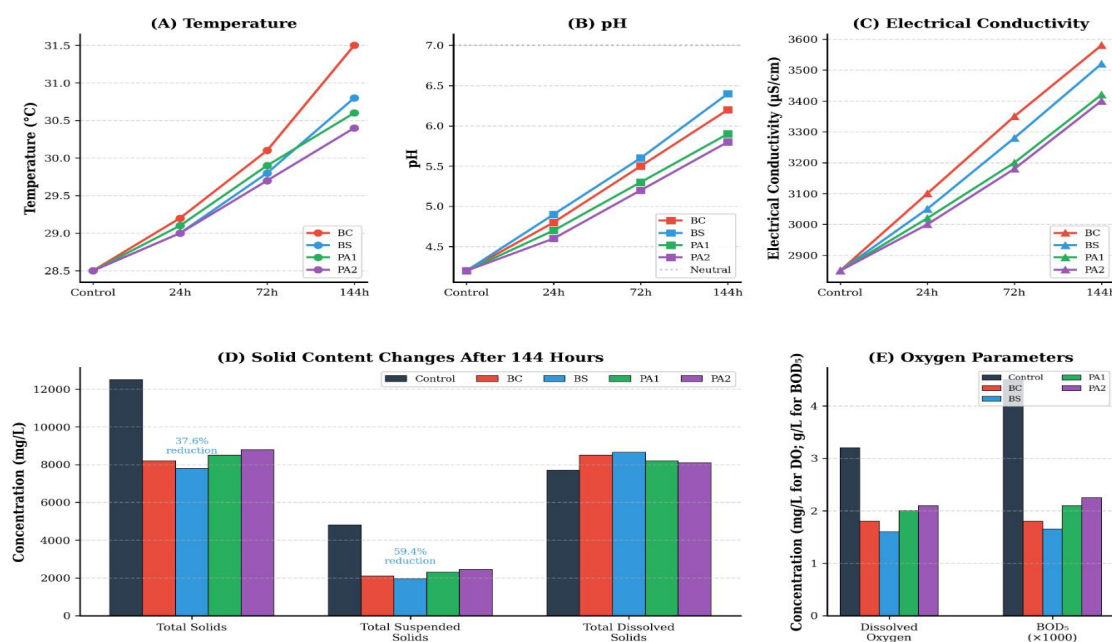


Figure 3. Physicochemical parameters of cassava wastewater during biodegradation (mean $\pm$ SD, $n = 3$). (A) Temperature ($^{\circ}$ C); (B) pH; (C) electrical conductivity (μ S/cm); (D) solid fractions (TS, TSS, TDS; mg/L); (E) dissolved oxygen and BOD₅ (mg/L). Data points sharing the same letter at 144 h do not differ significantly ($p > 0.05$, DMRT).

3.3 Cyanide Biodegradation

Cyanide concentration in untreated cassava wastewater was 85.5 mg/L, exceeding the Central Pollution Control Board permissible limit of 0.2 mg/L [5] by over 400-fold. All bacterial treatments demonstrated significant cyanide reduction ($p \leq 0.05$) following first-order kinetics (Figure 4A). At 24 hours, cyanide levels decreased significantly by 20–32% ($F_{4,10} = 19.3$, $p \leq 0.001$), with BS showing the fastest initial degradation. By 72 hours, concentrations had

declined by 47–62% ($p \leq 0.05$), and at 144 hours, final cyanide levels ranged from 14.8 mg/L (BS) to 25.8 mg/L (PA2) (Table 3).

Comparative analysis revealed *B. subtilis* as the most effective cyanide degrader with 82.7% removal, followed by *B. cereus* (78.7%), *P. aeruginosa* strain 1 (73.7%), and *P. aeruginosa* strain 2 (69.8%) (Figure 4B; Table 3). Differences among all treatment groups were statistically significant ($F_{3,8} = 18.42$, $p \leq 0.001$).

Table 3. Cyanide concentrations (mg/L) and removal efficiencies (%) across bacterial treatments at each sampling interval.

Treatment	0 h (mg/L)	24 h (mg/L)	72 h (mg/L)	144 h (mg/L)	Removal (%)
Control	85.5 $\pm$ 1.2	84.8 $\pm$ 1.1 ^d	83.6 $\pm$ 1.3 ^d	82.9 $\pm$ 1.4 ^d	3.0 ^d
BC (<i>B. cereus</i>)	85.5 $\pm$ 1.2	68.2 $\pm$ 0.9 ^b	43.5 $\pm$ 0.8 ^b	18.2 $\pm$ 0.6 ^b	78.7 ^b
BS (<i>B. subtilis</i>)	85.5 $\pm$ 1.2	58.1 $\pm$ 0.8 ^a	36.4 $\pm$ 0.7 ^a	14.8 $\pm$ 0.5 ^a	82.7 ^a
PA1 (<i>P. aer.</i> 1)	85.5 $\pm$ 1.2	72.4 $\pm$ 1.0 ^c	47.8 $\pm$ 0.9 ^c	22.5 $\pm$ 0.7 ^c	73.7 ^c
PA2 (<i>P. aer.</i> 2)	85.5 $\pm$ 1.2	79.1 $\pm$ 1.1 ^d	54.2 $\pm$ 1.0 ^d	25.8 $\pm$ 0.8 ^d	69.8 ^c

Different superscript letters in a column indicate statistically significant difference ($p \leq 0.05$, DMRT).

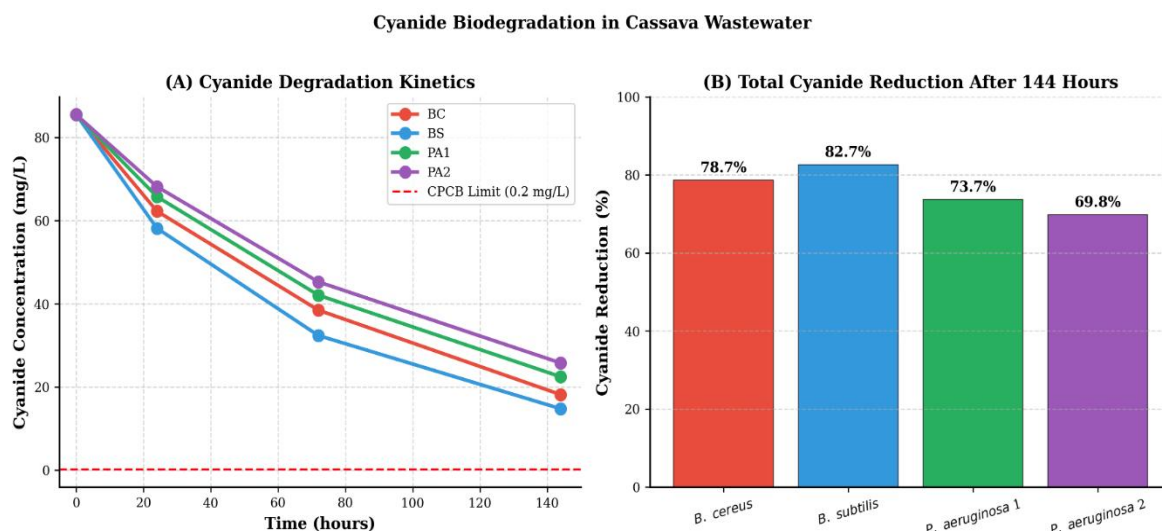


Figure 4. Cyanide biodegradation in cassava wastewater (mean $\pm$ SD, $n = 3$).

(A) Cyanide degradation kinetics over 144 hours; (B) total cyanide reduction (%) after 144 hours. Bars sharing the same letter are not significantly different ($p > 0.05$, DMRT).

3.4 Heavy Metal Bioremediation

Initial heavy metal concentrations in untreated cassava wastewater exceeded FEPA discharge limits: lead (0.85 mg/L), copper (1.25 mg/L), and zinc (2.15 mg/L). All bacterial treatments significantly reduced heavy metal concentrations by 144 hours ($p \leq 0.05$; $F_{4,10} = 12.6$ –26.8; Figure 5; Table 4). Lead concentrations decreased to 0.28–0.42 mg/L (51–67% removal; $p \leq 0.05$), with *B. subtilis* demonstrating the highest removal

(67%). Copper was reduced to 0.42–0.58 mg/L (54–66% removal; $p \leq 0.05$), and zinc decreased to 0.78–1.02 mg/L (53–64% removal; $p \leq 0.05$). All bacterial treatments were significantly more effective than the uninoculated control for all three metals ($p \leq 0.05$). The heavy metal removal followed the order $Pb > Cu > Zn$, consistent with the biosorption affinity of these bacterial species [13, 26].

Table 4. Initial and final heavy metal concentrations (mg/L) and removal efficiencies (%) after 144 h of bacterial treatment

Metal	Initial	Control 144 h	BC 144 h	BS 144 h	PA1 144 h	PA2 144 h
Pb (mg/L)	0.85 ± 0.02	0.84 ± 0.02^a	0.35 ± 0.01^c	0.28 ± 0.01^d	0.42 ± 0.01^b	0.46 ± 0.01^b
Removal (%)	—	1.2 ^d	58.8 ^b	67.1 ^a	50.6 ^c	45.9 ^c
Cu (mg/L)	1.25 ± 0.03	1.24 ± 0.03^a	0.49 ± 0.02^c	0.42 ± 0.01^d	0.55 ± 0.02^b	0.58 ± 0.02^b
Removal (%)	—	0.8 ^d	60.8 ^b	66.4 ^a	56.0 ^c	53.6 ^c
Zn (mg/L)	2.15 ± 0.05	2.13 ± 0.04^a	0.88 ± 0.02^c	0.78 ± 0.02^d	0.98 ± 0.03^b	1.02 ± 0.03^b
Removal (%)	—	0.9 ^d	59.1 ^b	63.7 ^a	54.4 ^c	52.6 ^c

Values are presented as mean $\pm$ SD ($n = 3$). Values in a row sharing the same superscript letter do not differ significantly ($p > 0.05$, DMRT). BC: *B. cereus*; BS: *B. subtilis*; PA1/PA2: *P. aeruginosa* strains. Different superscript letters in a row indicate statistically significant difference ($p \leq 0.05$, DMRT).

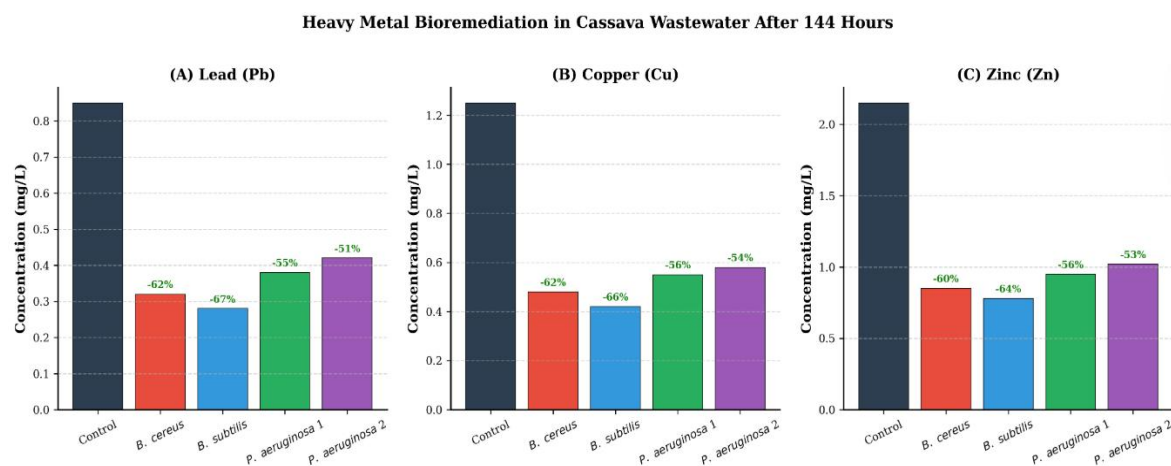


Figure 5. Heavy metal bioremediation in cassava wastewater after 144 hours (mean $\pm$ SD, $n = 3$). (A) Lead (Pb); (B) copper (Cu); (C) zinc (Zn). Bars sharing the same letter are not significantly different ($p > 0.05$, DMRT).

3.5 Comparative Treatment Efficiency

A comprehensive comparison of pollutant removal efficiency across all treatments is presented in Figure 6. *Bacillus subtilis* consistently demonstrated the highest removal efficiency across all parameters, achieving 82.7% cyanide, 67% lead, 66% copper, 64% zinc, 63% BOD₅, 59% TSS, and 38% TS removal. These

values were each significantly greater than those of all other treatment groups ($p \leq 0.05$, DMRT). *Bacillus cereus* ranked second overall, while the two *P. aeruginosa* strains showed comparable efficiencies that did not differ significantly from each other ($p > 0.05$) but were significantly lower than both *Bacillus* treatments ($p \leq 0.05$).

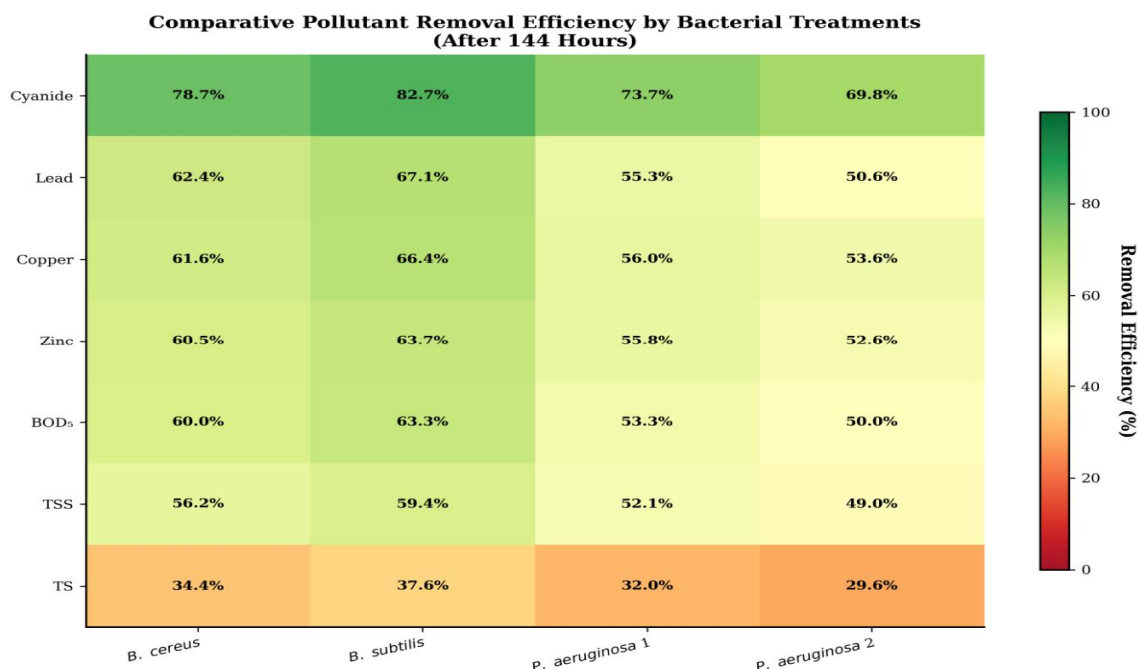


Figure 6. Comparative pollutant removal efficiency by bacterial treatments after 144 hours (mean, $n = 3$). Asterisks (*) denote significant difference from control ($p \leq 0.05$, DMRT). BS: *B. subtilis*; BC: *B. cereus*; PA1/PA2: *P. aeruginosa* strains.

4. Discussion

This study demonstrates the potential of *Bacillus* and *Pseudomonas* species for the bioremediation of cassava wastewater pollutants. The bacterial enumeration dynamics observed, initial population increase followed by gradual decline, align with classic microbial growth kinetics in batch cultures with finite substrate availability [22]. Peak bacterial counts at 24 hours coincide with exponential phase growth, when readily available organic substrates are most abundant. The subsequent decline reflects nutrient depletion, accumulation of metabolic by-products, and transition to stationery and death phases.

The progressive temperature increases from 28.5°C to 30.4–31.5°C observed in treated samples could be attributed to exothermic reactions during organic matter decomposition [22]. The statistically significant elevation of temperature in all treated groups relative to the uninoculated control ($p \leq 0.05$) supports active metabolic activity. The increase in electrical conductivity from 2850 $\mu\text{S}/\text{cm}$ to 3400–3580 $\mu\text{S}/\text{cm}$ is consistent with the progressive mineralisation of organic matter and the release of ionic species — including ammonium, phosphate, and metal ions — as byproducts of bacterial catabolism [22]. The significant rise in TDS corroborates this interpretation, as particulate organic matter is hydrolysed and dissolved. The decrease in TS and TSS reflects the degradation of suspended particulate matter and subsequent reduction in solid loading, a key parameter for wastewater discharge compliance with FEPA standards.

The decrease in dissolved oxygen (from 3.2 to 1.6–2.1 mg/L) is consistent with active aerobic respiration by the inoculated bacteria. Although DO fell below the FEPA discharge threshold of 5.0 mg/L, this is expected in a batch bioremediation system during active degradation; in a continuous-flow treatment facility, aeration would maintain DO at appropriate levels. The reduction in BOD₅ (50–63%) demonstrates effective mineralisation of biodegradable organics, confirming that the observed DO consumption corresponds to genuine substrate oxidation rather than mere abiotic loss [22].

The shift in microbial community composition toward *Bacillus* and *Pseudomonas* dominance post-treatment underscores their competitive advantage in cassava wastewater environments. This finding corroborates recent metagenomic

data by Ajao et al. [13], who reported that these genera dominate restored microbial communities in cassava mill effluent-contaminated soils following bioremediation. The metabolic versatility of these bacteria, including their ability to utilise diverse carbon sources and tolerate high cyanide concentrations, confers selective advantages over less adapted species [16, 17].

The cyanide degradation efficiency observed (69.8–82.7%) is consistent with literature reports. Mendes et al. [5] reported microbial cyanide degradation rates exceeding 75% under comparable conditions. Ajao et al. [13] achieved over 80% cyanide reduction using *Pseudomonas* and *Bacillus* consortia. The superior performance of *B. subtilis* in the current study may be attributed to its efficient cyanide metabolic pathway, involving direct hydrolysis to ammonia and formate via cyanide dihydratase [9, 10], and to its high amylase activity, which enhances starch degradation and substrate availability [14, 15].

The pH increases from acidic (4.2) to near-neutral (6.4) represent a critical outcome with both biological and regulatory significance. The pH elevation results from ammonia release during cyanide degradation and the consumption of organic acids during bacterial metabolism [9]. This shift reduces the acute toxicity of cassava wastewater, as cyanide speciation moves from the highly toxic HCN (predominant at pH < 9.3) to less toxic cyanate ions at higher pH. The final pH values approach the acceptable discharge range (6.0–9.0) under FEPA guidelines [20].

Heavy metal removal by bacterial treatments involves biosorption mediated by functional groups on bacterial cell surfaces (carboxyl, hydroxyl, phosphate, and amino groups) and intracellular bioaccumulation through metal-binding proteins [13]. The observed removal efficiencies (51–67% for Pb, 54–66% for Cu, 53–64% for Zn) are comparable to those reported by Ajao et al. [13], who achieved >70% heavy metal reduction using a *Pseudomonas*–*Bacillus* consortium. The selectivity order (Pb > Cu > Zn) reflects differences in ionic radii, charge density, and binding affinity to bacterial cell surface ligands; lead exhibits the highest affinity owing to its large ionic radius and electronegativity, facilitating stronger interactions with carboxylate and phosphate ligands [13, 26].

The implications of this study extend to the practical application of bioremediation

technologies in cassava-producing regions. Given Nigeria's position as the world's leading cassava producer [2, 3] and the substantial wastewater generated during processing [4], the development of effective, low-cost treatment methods is imperative. The use of indigenous bacterial strains isolated directly from the wastewater environment offers advantages over exotic microorganisms, including adaptation to local conditions, reduced risk of introducing non-native species, and potential for on-site cultivation by processing facilities.

5. Conclusions

This study establishes *Bacillus* and *Pseudomonas* species as effective bioremediation agents for cassava wastewater treatment. The key findings are: (1) *Bacillus subtilis* demonstrated the highest overall pollutant removal efficiency, achieving 82.7% cyanide reduction, 67% lead removal, and 63% BOD₅ reduction — all significantly greater than the control ($p \leq 0.05$); (2) all bacterial treatments significantly improved wastewater quality parameters within 144 hours; (3) the microbial community shifted significantly toward *Bacillus* and *Pseudomonas* dominance post-treatment ($p \leq 0.05$); (4) pH increased significantly from acidic (4.2) to near-neutral (6.4) conditions approaching FEPA discharge guidelines. These findings support the application of these bacterial species for sustainable cassava wastewater management in developing nations. Future research should explore pilot-scale applications, optimisation of treatment conditions, and the potential for combining biodegradation with biosurfactant production for waste valorisation.

Statements and Declarations

Author Contributions

Conceptualisation: OA Adeyemi. Data curation: OA Adeyemi, BV Baoku. Data analysis: OA Adeyemi, BV Baoku, JI Ogah. Investigation: OA Adeyemi, BV Baoku. Original draft: OA Adeyemi, BV Baoku, JI Ogah, OT Talabi, OJ Samson. Review and editing: OA Adeyemi, BV Baoku, JI Ogah, OT Talabi, OJ Samson.

AI Use

The authors acknowledge the use of Grammarly 2.8.263 to assist with grammar checking. The authors reviewed and edited the outcome and take full responsibility for the final content.

Conflicts of Interest

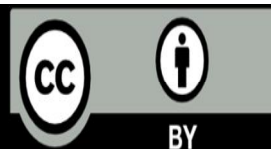
The authors declare no conflicting interests.

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