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Evaluation of the Potential of Immobilized Cyanide-Degrading Bacteria for the Bioremediation of Cassava Mill Effluent

Ajao[1,*], Abdullahi Taiwo, Jimoh-Hamza[2], Oluwabukola Kudirat, Aborisade[1], Wakili Tope and Jimoh[1], Fausat Abimbola

[1]*Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, Nigeria;*[2]*Department of Biological Sciences,Faculty of Natural and Applied Sciences, Al-Hikmah University, Ilorin, Nigeria.*

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Abstract

This study aimed to assess the feasibility of employing indigenous cyanide-degrading bacterial strains for the treatment of cassava mill effluent (CME) in Nigeria, a hazardous waste material posing significant public health risks. The physicochemical properties and heavy metal concentrations in CME were determined through standard methodologies. Cyanide-degrading bacteria were isolated, characterized, and identified using molecular techniques. These strains were immobilized within a porous network of cross-linked biochar, confirmed through SEM imaging, and evaluated alongside free cells for CME bioremediation. Multiple variables were examined to assess the effluent's pollution load, including pH, electrical conductivity (EC), chemical oxygen demand (COD), biochemical oxygen demand (BOD5), total dissolved solids (TDS), nitrate (NO3), phosphate (PO4), and cyanide levels, falling within respective ranges of (4.00–4.02), (2394–2618 S/cm), (985–1219 mg/L), (1556–1667 mg/L), (3459–3705 mg/L), (2709–2812 mg/L), (251-311 mg/L), (87.25–118 mg/L), and (25.00–28 mg/L). Copper was the most prevalent heavy metal in the effluent, while chromium was detected at the lowest levels, with mean values of 10.00-12.39 mg/L and 0.00-0.27 mg/L, respectively. The study revealed severe contamination of the effluent, above the Federal Environmental Protection Agency (FEPA) threshold limit. The 16S rDNA of the cyanide degrading bacteria were deposited at the NCBI database and the following GenBank accession numbers were assigned (MK71248O, MK71281, MK712482, and MK712483) for *Pseudomonas putida*, *Bacillus subtilis*, *Alcaligenes faecalis* and *Leuconostoc mesenteroides* respectively. Results of the bioremediation approach demonstrated a substantial reduction in physicochemical parameters and heavy metal contents, indicating a promising biological treatment strategy to mitigate CME's adverse effects on public health. In conclusion, despite elevated physicochemical parameters and heavy metal concentrations in CME, this study offers a promising avenue for employing biological treatments to mitigate environmental and public health impacts. Further research and application of this technique hold substantial potential for cassava mill effluent management in Nigeria and other regions facing similar challenges.

Keywords: Bioremediation; Cassava; cyanide; immobilization; physicochemical; heavy metals

1. Introduction

According to Afuye and Mogaji (2015), cassava (Manihot esculenta Crantz) is regarded as a crucial food crop, particularly in African nations, with Nigeria serving as a notable example. Cassava production and processing are largely dominated by a number of small-scale farmers as their source of livelihood (Izah *et al*., 2017a-c). Nigeria was ranked as the top cassava producer in the world in 2016 with a production of 5, 7134,478 tonnes (Izah *et al*., 2018). Moreover, Cassava is the next significant contributor to gross domestic product to the Nigeria economy after petroleum (Jickson *et al*., 2013).

One of the procedures in the processing of cassava is cassava milling, which generates a substantial amount of hazardous waste typically disposed of untreated into nearby farmland and water bodies, posing a risk to public health (Obueh and Odesiri-Erunteyan, 2018; Izah *et al*., 2018). Additionally, the cassava processing industries produce a significant volume of toxic wastewater, constituting a serious environmental threat to both soil biota and aquatic life. This wastewater contains cyanogenic glycosides, primarily linamarin and lotaustralin, which constitute the majority of the hazardous and acidic elements in cassava mill effluent (CME) (Okunade and Adekalu, 2014). According to earlier studies (Oluwafemi *et al*., 2011; Ezeigbo *et al*., 2014; Eboibi *et al*., 2018; Izah, 2018), this wastewater has the potential to cause toxicological effects and have an impact on the biodiversity of soil biota and vegetation if it is not properly treated. Cassava processing has always been regarded with a reputation of a major environment pollutant. Expansion of cassava from subsistence to commercial production, and to the second largest crop production in the country, raised many concerns especially in its relation to the waste management.

Indiscriminately releasing waste products from Cassava processing factories into the environment has been a common practice, thereby polluting the agricultural soil,

* Corresponding author. e-mail: abdullahi.ajao@kwasu.edu.ng.

surface and underground waters (Ezeigbo *et al.,* 2014) induces changes in soil health index such as physicochemical properties, microbial community, building up of toxic metals, and numerous factors associated with plant development (Patrick *et al*.,2011: Chinyere *et al*., 2013: Izah *et al*., 2017). As such, these wastes could even be more difficult to handle in future with the scale-up in the cassava production and processing (Izah *et al*., 2017: Eneriviofi *et al*., 2017).

Considering cassava's economic and agricultural importance, along with its contribution to enhancing food security in Nigeria, it is essential to prioritize the management and processing of cassava waste in urban areas of the country (Oluwatosin *et al*., 2017). Most of the available physicochemical methods for the removal of cyanide are not satisfactory in terms of the cost, toxicity and generation of hazardous chemical end products. Given the limitations of physicochemical methods, there is a pressing need for a robust and economically viable strategy for treating cyanide-contaminated natural ecosystems (Kandasawy et al., 2015; Akinpelu et al., 2017). The degradative capacity of microbes to control environmental pollution has been explored (Bioremediation). Apparently, bioremediation strategy has become method of choice due to its inexpensive, environmentally friendly and efficient nature (Ugochukwu *et al*., 2014; Sarkar *et al*., 2016; Oluwatosin *et al*., 2017; Gupta *et al*., 2017). The study introduces novelty through the utilization of immobilized cells, a method not previously applied for the bioremediation of cassava mill effluents due to its unique design and methodology. To the best of our knowledge, this work is pioneering in introducing such an approach for processing cassava mill effluents. These findings represent the first preliminary evidence for this innovative solution, and this would be of great advantage in terms of its simplicity and appropriate choice to explore for the waste treatment and management that can be adopted by small-scale farmers to detoxify contaminated CME before being released into the environment.

2. Materials and Methods

2.1. Study Area

The study areas are the selected Cassava processing Factories located within Ilorin Metropolis, the capital of Kwara State, located between the latitude 8º5′-10º4′N and longitude 4º55′-6º5′E (NPC, 2006) as shown in Fig. 1. The region lies in the rainforest zone in the South and Guinea Savannah in the North Zone. The average temperature and rainfall are within (27 ^{O}C - 35 ^{O}C) and (1,000 – 1500 mm) respectively. The total land surface area is 35,705 square kilometer (Sqm). Kwara State is Cassava, Millet, Yam, Cowpea producing area due to the vegetation cover, climatic condition and soil types that support the cultivation.

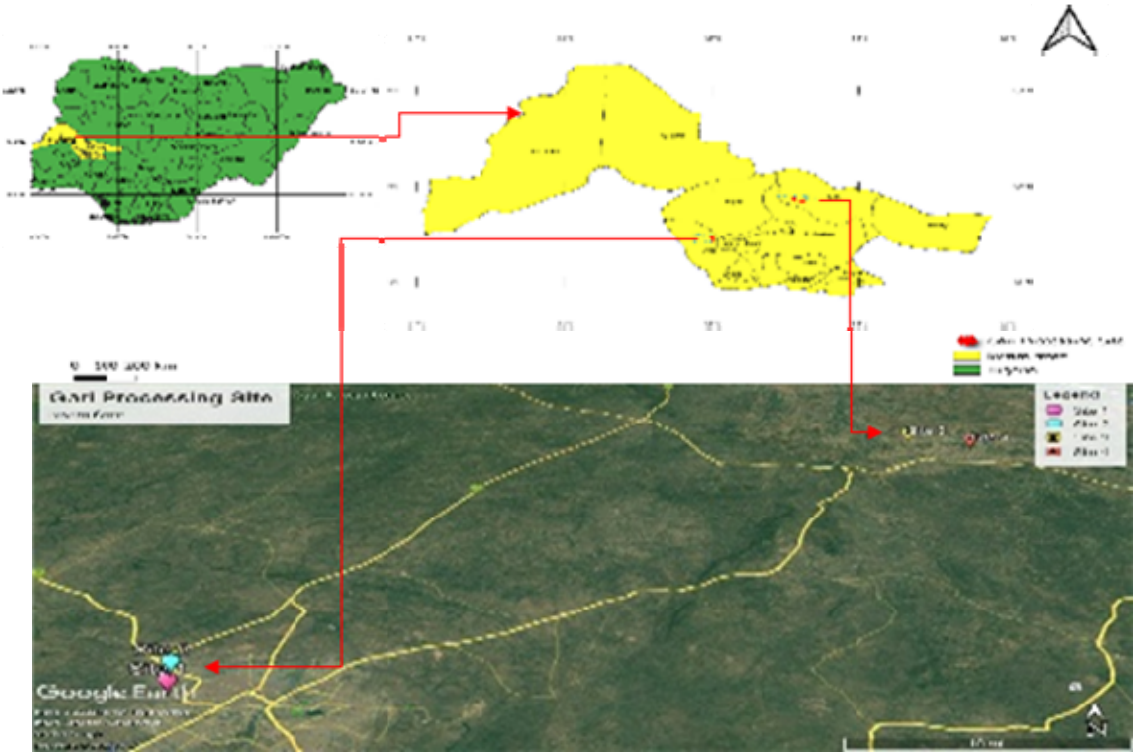

Figure 1.Map of the sampling sites

"Footnote: This map displays four distinct sites representing various Gari Processing Factories from which cassava wastewater samples were collected."

2.2. Sample Collection

This investigation adhered to the methodology outlined by Oljiira *et al*. (2018). Wastewater samples were obtained from the selected cassava processing factories located in the Ilorin metropolis. One-liter samples were aseptically collected from beneath the hydraulic press into clean plastic bottles, which had been cleaned with detergent and rinsed with acid. The samples were then quickly transported to the lab and kept cool at 4 °C in a cooler with ice blocks. To establish a baseline for the assessment in this study, the Agoro *et al*. (2018) method was used to evaluate the physicochemical properties of the wastewater samples.

2.3. Determination of Physicochemical Characteristics of CME

The methodology outlined by Oljiira *et al*. (2018) was employed to examine and measure the physicochemical properties and heavy metal concentrations in CME. TS, TSS, and TDS were determined using the Gravimetric method. The pH was assessed using a pH meter (Model HI9024, HANNA Instrument, UK), and EC was measured with an Oyster conductivity meter during the grab-sampling procedure conducted at the sampling site. Nitrate (ultraviolet spectrophotometric screening methods), Sulfate (Turbidimetric method), Phosphate (Vanadomolybdophosphoric acid) Chloride (argentometric), were determined using standard procedure (APHA, 1998). The determination of COD was performed using a closed reflux method. BOD_5 was measured from the dissolved oxygen content estimated in a BOD5 bottle at 20 °C for 5 days before and after incubation. The CME samples were digested in concentrated nitric acid before being diluted, centrifuged, and filtered using 0.45 m pore size membrane filter. The levels of heavy metals found in wastewater sample were determined using atomic absorption spectroscopy (AAS), including lead, chromium, nickel, copper, manganese, and zinc with a Perkin-Elmer Analyst 300 instrument.

2.4. Determination of the Cyanide Contents in Cassava wastewater

The quantification of cyanide content in cassava wastewater followed the methodology outlined by Eke-Emezia *et al*. (2022) study. In this approach, 2 ml of an alkaline picrate solution was combined with 2 ml of the cassava wastewater sample. The resulting mixture underwent a 10-minute incubation period at 60 °C within a water bath. Following incubation, an absorbance measurement was performed on the resulting deep orange-colored solution using a U-V Spectrophotometer (SHIMADZU UV-1800). The cyanide concentration was determined by extrapolating the values from a previously established potassium cyanide curve, serving as a reference standard.

2.5. Isolation of Cyanide-Degrading Bacteria Using Enrichment Techniques

Cyanide-degrading bacteria were isolated from the soil samples impacted with the cassava mill effluents obtained from the cassava processing factories. These samples were cultivated and enriched in a growth medium that included glucose as the carbon source and KCN as the nitrogen source. The procedure for preparing the medium and isolating bacteria was carried out in accordance with Kandasawy *et al*. (2015) method. The bacterial colonies suspected of degrading cyanide were streaked onto agar slants and then classified based on cultural morphology and biochemical test

2.6. Molecular Identification of Bacterial Isolates.

The genomic DNA was extracted from pure colonies of cyanide-degrading bacteria and suspended in lysis buffer supplemented with Proteinase K to facilitate the extraction process. Subsequently, amplification was performed using universal primers in a thermal cycler, following the conditions specified in Table 1, using established according to the protocol described by Bhutia et al. (2021).

Table 1.16S Primer Sequences

Name of Primers	Target	Sequences (5'-3')
16-518F	Universal primers	5' – CGCTTGTTGATTTGCTGCTGTTCGC – 3'
16S-800R	Universal primers	5-TACCAGGGTATCTAATCC-3
PCR Conditions	Denaturation at 92°C, Annealing at 54°C, and Extension at 72°C,	25 cycles

After purification with the QIA quick PCR purification kit from Qiagen, USA, 1% (w/v) agarose gels were used to evaluate the PCR product. Subsequently, 16S rRNA gene partial sequencing was conducted using Applied Biosystems' BIG-DYE terminator kit for the ABI 310 Genetic Analyzer. Homologous bacteria were identified, and bacterial sequences were aligned using the BLAST Search program from the National Center for Biotechnology Information. To construct the phylogenetic tree, the neighbor-joining technique was employed. The obtained bacterial sequences were deposited into the Genbank of the National Center for Biotechnology Information (NCBI) and assigned accession numbers.

2.7. Preparation of Biochar as a carrier for the cell Immobilization

The preparation of biochar was done according to the method described by Chen and Chen (2009). About 5g of the Activated cow dung was utilized as the biochar material and heated to a temperature of 500 ^{0}C for duration of 4 hours. After cooling for 12 hours, the biochar was then ground and sieved into a fine particle (2mm NO. 60 U.S. Std. mesh). The resultant powder was kept for later use in an airtight container.

2.8. Preparation of Immobilized consortium on carrier

The pre-sterilized biochar, sodium alginate and calcium chloride at 121 °C for 15 mins was used to immobilize the consortium of highest cyanide degrading bacteria as described by (Chen *et al*., 2017). In 100 mL of the mixed culture of the 0.1 at OD_{600} of the cell density of the bacteria, 5 g of the biochar was added and vortexed for 15 minutes. Then, the mixture was added to and mixed for 15 minutes with a 2% (w/v) solution of sodium alginate. The resultant mixture, an adsorption-carrier suspension, was

extruded through a 20 mL syringe and introduced into a sterilized solution of 2% (w/v) calcium chloride to undergo cross-linking for 12 hours, resulting in the formation of spherical beads. The immobilized microsphere was washed with sterile distilled water and subsequently stored in a similar environment.

2.9. Preparation of Microsphere for SEM

The entrapped cells within the porous network of the cross linked biochar characterized using scanning electron microscopy (SEM; KYKY-2800B) as follows: The immobilized cells were fixed for 2 hours with 2.5% glutaraldehyde while the bio-carriers were dried and coated with gold using the method of CO_2-critical point drying. According to protocol, an electron microscope was used to examine the morphology of immobilized bacterial cells.

2.10. SEM Focusing of Microsphere

The samples were mounted onto an aluminum holder stub by utilizing a double sticky carbon tape. Subsequently, gold was applied to the sample, and it was electrically grounded. In order to prevent confusion between samples, the sample stub was marked or inscribed since it can be difficult to distinguish between similar samples in the SEM. Before being loaded into the SEM holder, the sample was completely dried for a minimum of three hours in a drying oven at 60 °C as described by Khashei *et al*. (2018). The sample was focused and magnified as per standard operating procedure with the holder in place on the microscope stage.

2.11. Bioremediation of Cassava Mill Effluent (CME)

Free cells and Immobilized cyanide degrading bacterial Consortium were selected for the bioremediation strategy used for the treatment of CME using modified method of Sonune and Garode's (Sonune and Garode, 2009). The cell density was adjusted to 0.5 at $O.D_{600}$ and inoculum size of 10 % was inoculated into 250 mL Enlenmeyer flask containing 90 mL presterilized cassava wastewater (free cells). Afterwards, 10 ml of the microsphere of immobilized cells was added into 90 ml of presterilized cassava mill effluent in the flasks. Simultaneously, control flasks contained only sterilized Cassava Mill effluent. Each of the flasks was placed on an orbital shaker, where they were maintained at a temperature of 30 °C and rotated at a speed of 120 revolutions per minute for duration of 12 days. All samples were withdrawn at every 72 hrs interval, centrifuged at 4000 rpm for 20 mins and determined the variables in triplicates. The efficiency of the treatment strategy was evaluated by the decrease in the physicochemical parameters and the concentration of heavy metals over the course of treatment.

2.12. Statistical Analysis

One way, variance analysis (ANOVA) has been used to compare the mean of the parameters evaluated. Using the Social Sciences Statistical System (SPSS) Duncan Multiple Range Analysis at $p<0.05$ between the mean values of the calculated parameters.

3. Results

The physicochemical parameters of the sampled Cassava Mill Effluent were determined experimentally, with mean values falling within the ranges of pH (4.00-4.20), EC (2550-2580 µs/cm), BOD5 (980-1215 mg/L), COD (1550-1660 mg/L), Phosphate (87-118 mg/L), and Nitrate (300-310 mg/L). The cyanide content ranged from (28.74-25.00 mg/L), all of which exceeded the permissible limits set by Fedral Environmental Protection Agency (FEPA) (Table 2). The statistical analysis revealed that the mean values obtained from each of the sampling stations were not significantly different, according to the Duncan multiple range tests ($p<0.05$).

Total heterotrophic bacterial counts were (2.7 log CFU/g) and (6.0 log CFU/g) in the Cassava Mill Effluent and the soil impacted by the effluent, respectively, while the heavy metals detected were Lead (0.53-1.15 mgL^{-1}), Chromium (0.16-0.27 mgL^{-1}), Nickel (1.86-2.89 mgL^{-1}), and Copper ions (10.00-12.39 mgL^{-1}) (Table 3).

The four highest cyanide-degrading bacterial strains were tentatively identified as *Pseudomonas* sp, *Lactobacillus* sp, *Bacillus* sp, and a member of *Enterobacteriaceae* for HM1, HM2, HM5, and HM2a, respectively. The 16S rRNA gene sequences of these strains were deposited in the NCBI database under the accession numbers MK712480, MK712481, MK712482, and MK712483, respectively. Strain HM1 showed the highest homology (100%) with *Pseudomonas putida* strain NBRC14164, while strain HM2a was closely related to *Alcaligenes feacalis* strain IAM (100%). Strain HM2 was most closely related (100%) to *Lactobacillus mesenteroides* subsp jonggjibkimchii strain DRC1506, and strain HM5 showed the highest homology with *Bacillus subtilis* strain IAM 12118. These cyanide-degrading bacterial isolates clustered with members of the genera *Pseudomonas, Alcaligenes, Leuconostic*, and *Bacillus* (as shown in Fig. 1).

3.1. SEM of Bio-carriers of calcium Alginate and immobilized Bacterial Consortium

Plate 1 depicts scanning electron microscope (SEM) photomicrographs of calcium alginate that have not been subjected to the attachment of cyanide-degrading bacterial cells. Plate 2 displays a photomicrograph of a consortium of immobilized cells attached to the surface of the biocarrier, which is composed of calcium alginate.

3.2. Physicochemical Parameters of Cassava Wastewater Analysis after 12 Days Treatment

Table 4 presents the physicochemical parameters of the Cassava Mill Effluent (CME) before and after undergoing treatment. Both Free Bacterial Cells (FBC) and Immobilized Cell Consortium (ICC) treatments resulted in a significant reduction in all physicochemical parameters. The analysis of variance revealed that the ICC treatment had the greatest impact on the treatment compared to the control set-up. Conversely, FBC-treated wastewater exhibited minimal removal of heavy metals, whereas immobilized cells demonstrated effective remediation results for heavy metals. Analysis of variance revealed significant differences among the three treatments, with significant differences observed in Pb^{2+} (0.06 ± 0.37 mg/L), Cr^{6+} (00.00 mg/L), and Ni^{2+} (0.02 ± 0.01 mg/L) when compared to the control and FEPA limit. Both treatments were unable to reduce Cu^{2+} to the FEPA limit value specified in Table 5.0.

Table 2. Physicochemical and Bacteriological Characteristics of the Wastewater from Cassava Processing Factories in Ilorin Metropolis

Parameters	A	B	C	D	FEPA (mg/l).	C. S	5.9[a]	5.7[a]	5.8[a]	6.0[a]
						WW	2.4[a]	2.7[a]	2.3[a]	2.3[a]
PH	4.00 ± 0.03 [a]	4.02 ± 0.00 [a]	4.01 ± 0.57 [a]	4.00 ± 0.00 [a]	6-9					
EC(us/cm)	2555 ± 7.07[ab]	2394 ± 5.66[b]	2618 ± 3.54 [a]	2586 ± 1.41 [ab]	1000					
BOD (mg/l)	1107 ± 3.54[b]	1219 ± 1.41 [a]	985 ± 2.12 c	1003 ± 071 c	50					
COD (mg/l)	1556 ± 2.83[b]	1667 ± 2.83 [a]	1577 ± 2.12 [b]	1602 ± 1041 [ab]	160					
TSS (mg/l)	3470 ± 3.54[bc]	3705 ± 2.12 [a]	3556 ± 2.12 [b]	3459 ± 5.66 [bc]	30					
TDS (mg/l)	2751 ± 3.54[b]	2812 ± 4024 [a]	2707 ± 5.66 [b]	2789 ± 1.41 [a]	2000					
PO_4 (mg/l)	106 ± 0.71[a]	97 ± 0.71 [a]	87.25 ± 0.35 [a]	118 ± 0.00 [a]	5.0					
NO_3(mg/l)	302 ± 1.45[a]	251 ± 0.71 [b]	287 ± 2.12 [ab]	311 ± 1.41 [a]	20					
Cyanide (mg/l)	25.00 ± 0.57[a]	28.74 ± 0.45 [a]	28.70 ± 0.00 [a]	27.11 ± 0.14 [a]	0.2					
C.S. (log CFU/g)	5.9 ± 0.00[a]	5.7 ± 0.00 [a]	5.8 ± 0.00[a]	6.0 ± 0.02[a]						
WW (log cfu/ml)	2.4 ± 0.00[a]	2.7 ± 0.05[a]	2.3 ± 0.00[a]	2.3 ± 0.00[a]						

Keys: A-Alagbado; B-Adeta; C- Pakata; D-Okelele.C.S- Contaminated soil; WW- Wastewater; EC-Electrical Conductivity; BOD-Biochemical Oxygen Demand;COD- Chemical Oxygen Demand; TSS-Total Suspended Solid; PO_4-Total Phosphate; NO_3-Total Nitrate; TDS-Total Dissolved Solid, CN^{-}- Cyanide contents.

Turkey's test, columns (with a sample size of n = 3) marked with distinct lowercase letters indicate statistically significant variations ($P < 0.05$) among the different treatments.

Table 3. Heavy Metal Concentrations of the Wastewater from Cassava Processing Factories in Ilorin Metropolis (mg/mL) (Mean ± SEM; n = 3).

Heavy metals	A	B	C	D	FEPA
Cr^{2+}	0.18 ± 0.00[a]	0.27 ± 0.00a	0.00 ± 0.00a	0.16 ± 0.00a	0.5
Pb^{2+}	0.54 ± 0.01b	1.15 ± 0.00a	0.67 ± 0.01b	0.83 ± 0.08b	0.5
Ni^{2+}	2.89 ± 0.03a	2.05 ± 0.07b	1.86 ± 0.01bc	2.10 ± 0.00b	1.0
CU^{2+}	12.39 ± 0.04a	10.48 ± 0.03b	10.00 ± 0.00b	11.89 ± 0.02a	1.5

Keys : Pb^{2+}-Lead ion, Cr^{6+}-Chromium ion, Ni^{2+}- Nickel ion Cu^{2+}- Copper ion

Alagbado; **B-**Adeta; **C**-Pakata; **D**-Okelele.

* Turkey's test, columns (with a sample size of n = 3) marked with distinct lowercase letters indicate statistically significant variations ($P < 0.05$) among the different treatments.

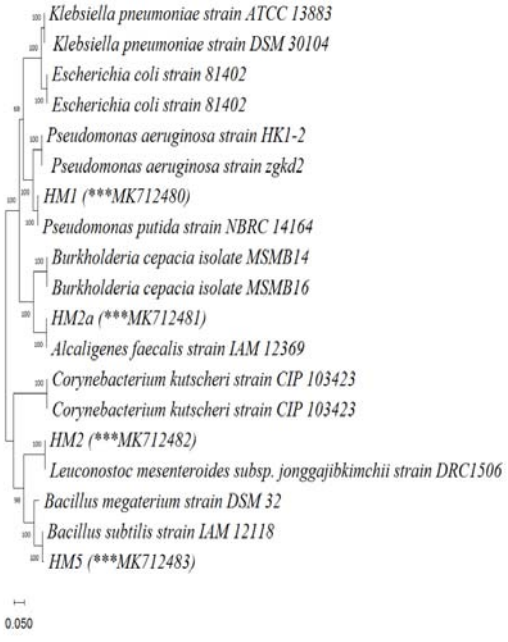

Figure 1. The phylogenetic tree of cyanide-degrading bacterial isolates. The numbers in parenthesis and asterisks indicate the GenBank accession numbers.

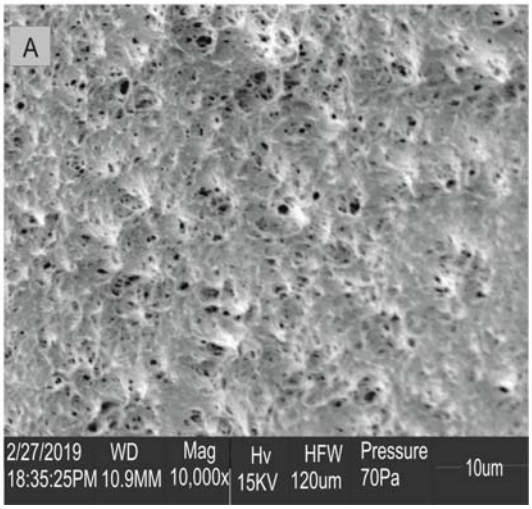

Plate 1. Biocarrier-calcium alginate in SEM photomicrography without the cell consortium

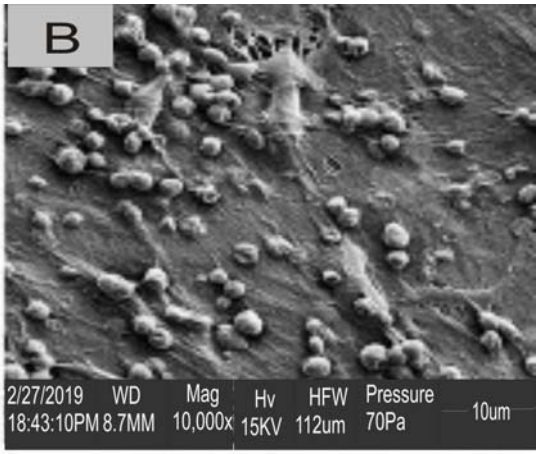

Plate 2. SEM photomicrograph of the immobilized cyanide-degrading bacterial cells consortium on the surface of the biocarrier of calcium alginate.

3.3. Pollution Reduction Efficiency (%) of FBC and ICC

The results depicted in Figure 2 demonstrated the effectiveness of the immobilized consortia (FBC and ICC) in reducing the levels of pollutants (physicochemical parameters) during the treatment process. The immobilized cells exhibited substantial removal efficiencies, as indicated by the high percentage removal values. The removal percentages for various parameters were higher for ICC compared to FBC. Specifically, for BOD5 and COD, ICC achieved removal percentages of 89.01% and 87.10%, while FBC achieved 71.10% and 67.10%, respectively. For NO_3 and TDS, ICC showed removal percentages of 68.00% and 59.60%, whereas FBC achieved 60.00% and 43.20%, respectively. In the case of PO_4 and cyanide concentrations, ICC demonstrated removal percentages of 70.00% and 84.25%, while FBC achieved 47.17% and 60.62%, respectively.

3.4. Heavy Metal Removal Efficiency (%) of Immobilized Cell Consortium and Free Bacteria Consortium

Table 4 summarizes the percentage efficiency of two treatment methods: immobilized cell consortium (ICC) and free bacterial cells (FBC), in addressing heavy metal contamination. Both treatments showed significant reductions in heavy metal concentrations. Specifically, for Pb^{2+} and Cr^{6+}, ICC achieved reductions of 56.60% and 88.68%, while FBC achieved 16.67% and 100%, respectively. For Ni^{2+} and Cu^{2+}, ICC demonstrated reductions of 62.37% and 99.65%, while FBC achieved 30.42% and 73.7%, respectively. These findings highlight the effectiveness of the immobilized cell consortium, particularly in reducing heavy metal concentrations, indicating its efficacy as a treatment process.

Table 4: Physiochemical Characteristics of the Treated Wastewater from Cassava Processing Factory (Means ± SD; n=3)

Treatment	pH	EC (mS/cm)	BOD (mg/l)	COD (mg/l)	TSS (mg/l)	PO_4 (mg/l)	NO_3 (mg/l)	TDS (mg/l)	CYANIDE (mg/l)
FBC	6.20 ± 0.55^{ab}	2770 ± 6.35^{ab}	310 ± 2.9^{b}	510 ± 1.87^{b}	1550 ± 9.4^{b}	56 ± 1.6^{b}	122 ± 20^{b}	990 ± 7.20^{b}	10 ± 1.10^{b}
ICC	7.50 ± 0.00^{a}	2830 ± 12.8^{a}	120 ± 2.1^{c}	200 ± 1.00^{c}	1100 ± 8.0^{c}	32 ± 1.1^{c}	96 ± 2.66^{c}	710 ± 3.50^{c}	4 ± 0.11^{c}
Control	4.40 ± 0.0^{b}	24500 ± 10^{b}	1000 ± 3.4^{a}	1450 ± 5^{ab}	3400 ± 12^{a}	93 ± 3.0^{ab}	290 ± 2.1^{a}	2670 ± 16^{a}	23.90 ± 1.0^{a}
FEPA Limit	6-9	1000	50	160	30	5	20	2000	0.2

Turkey's test, columns (with a sample size of n = 3) marked with distinct lowercase letters indicate statistically significant variations ($P < 0.05$) among the different treatments.

BOD: Biochemical Oxygen Demand; FBC :Free Bacterial Consortium; COD: Chemical Oxygen Demand; TSS: Total Suspended Solid; ICC: Immobilized Cell Consortium; TDS: Total Dissolved Solids; CN^-:Cyanide, PO_4 :Phosphate; NO_3:Nitrate; EC: Electrical Conductivity and TSS:Total suspended solids; FEPA: Federal Environmental Protection Agency.

Table 5. Heavy Metals Concentration of the Treated Wastewater from Cassava Processing Factory (mg/L)

Treatment	Pb^{2+}	Cr^{6+}	N_i^{2+}	Cu^{2+}
FBC	0.22 ± 0.01^{b}	0.15 ± 0.01^{a}	1.40 ± 0.57^{b}	8.58 ± 0.04^{b}
ICC	0.06 ± 0.37^{c}	0.0 ± 0.00^{b}	0.02 ± 0.01^{c}	3.24 ± 0.01^{c}
Control	0.51 ± 0.01^{a}	0.16 ± 0.01^{a}	2.87 ± 0.01^{a}	12.33 ± 0.03^{a}
FEPA	0.5	0.5	1.0	1.5

Key :FBC – Free Bacterial Consortium, ICC – Immobilized Cell Consortium, Pb^{2+} - Lead ion, Zn^{2+} - Zinc ion, Cu^{2+} – Copper ion, Ni^{2+} – Nikel ion, Mn^{2+} – Manganese, Cr^{6+}– Chromium ion .

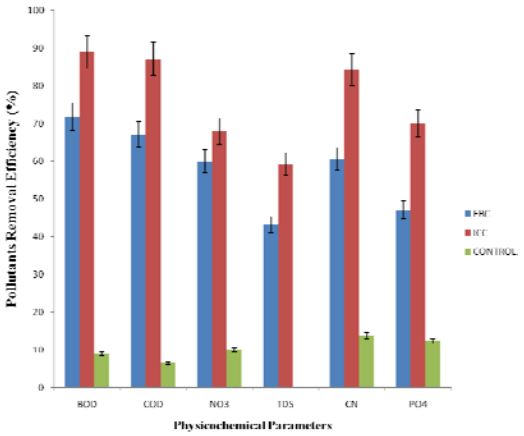

Figure 2. Pollutant Removal Efficiency (%) of FBC and ICC

Keys: FBC –Free Bacteria Consortium, ICC- Immobilized Cell Consortium, BOD-Biochemical Oxygen Demand, COD-Chemical Oxygen Demand, TSS-Total Suspended Solid, PO^{4-}Total Phosphate,NO^{3-}Total Nitrate, TDS-Total Dissolved Solid, CN^--Cyanide

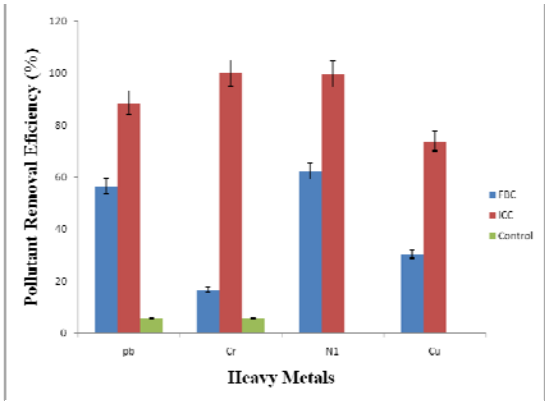

Figure 3.: Heavy Metal Removal Efficiency (%) of Free Bacteria Consortium and Immobilized Cell Consortium
Keys : Pb^{2+} -Lead ion, Cr^{6+} -Chromium ion, Ni^{2+} - Nickel ion Cu^{2+} -Copper ion

4. Discussion

The presence of cyanide, high values of the physicochemical parameters, the effluents' acidic pH, and the presence of heavy metals have earlier all been linked to their toxicity obtained in the current study (Izah, 2018). The pH of the cassava wastewater, which was in the range of 4.00 to 4.20, indicated the acidic nature of the cassava mill effluent (CME). This concurs with the findings of Uzochukwu *et al.* (2001), who noted comparable pH ranges. Nevertheless, aquatic life is reportedly impacted by pH levels between 3.5 and 4.5 (Adarsh and Mchantesh, 2006). Similarly, the mean values of EC, phosphate and TDS recorded in the CME exceeded the established regulatory limit.

The BOD5 and COD values derived from the cassava mill effluent (CME) exceeded the allowable threshold, with readings ranging from 985 to 1219 mg/l and 1556 to 1667 mg/l, respectively (Adewumi *et al*., 2006; Rum-Rukeh, 2016). Moreover, cyanide levels in the CME ranged from 25.00 to 28.74 mg/L, exceeding the allowable limit, as reported by Obueh and Odesiri-Eruteyan (2018). This result was in line with earlier research that showed cassava mill effluent to contain high levels of cyanide. (Okafor and Nwankwegu, 2016; Adewumi *et al*., 2016).

Furthermore, the soil polluted with the CME had high mean values of heavy metals, consistent with previous studies (Aiyegoro *et al*., 2007; Osakwe, 2012; Izah *et al*., 2017). The hazardous waste generated from cassava milling operations poses a significant risk to the environment, particularly soil and surface water, due to the presence of cyanide, high levels of physicochemical parameters, the acidic pH of the effluent, and the presence of heavy metals (Izah, 2018).

The primary sources of heavy metals in cassava mill effluent include agricultural machinery and appliances, soil biosorption, tool wear during harvesting and peeling, metal milling machine parts, and heavy metal contamination from water used in washing or processing. Heavy metal toxicity of this nature could potentially impact soil fertility and the population and diversity of soil biota (Ayansina and Olubukola, 2017). The bacterial strains designated as HM1, HM2a, HM2, and HM5 isolated in this study are closely related to *Pseudomonas putida, Enterococcus faecalis, Leuconostoc mesenteroides jonggajibkimchii,* and *Bacillus subtilis*, respectively, as determined by 16S rDNA sequencing and phylogenetic analysis (as shown in Figure 1). Those taxa showed 100 % similarity in their 16S rRNA-gene sequences to those of GenBank type species. The literature has described the cyanide-degrading ability of *P. putida* (Chatpawala *et al*., 1998; Kandasawy *et al*., 2015).Similarly, several other authors have also reported *Bacillus and Pseudomonas* in their study from cassava mill effluent (Obueh and Odesiri-Erunteyan, 2018: Enerijiofi *et al*., 2017).

The findings of this bioremediation study are consistent with the earlier studies (Ajao *et al*., 2011; Eneriviofi *et al*., 2017; Obueh and Odesiri-Erunteyan, 2018), despite differences in wastewater compositions. This investigation demonstrates the efficacy of both encapsulated and planktonic bacterial species in treating cyanide-laden wastewater, achieving substantial reductions in physicochemical parameters and heavy metal levels below regulatory thresholds. These findings represent an initial step in substantiating the practicality of this innovative approach. Notably, the immobilization of cells is a novel technique, previously unexplored in this context.

In both the free and immobilized treated wastewater, the pH of the cassava mill effluent increased from an initial value of 4.00 to 6.20 and 7.50, respectively; following 12 days of treatment, it fell within the permissible limit of 6-9 in both treatments as recommended for effluent to be discharged (FEPA, 1991). Several authors (Chuvudhary *et al*., 2011; Gaikwad *et al*., 2014; Izah *et al*., 2017) have reported comparable results.

Before treatment, the initial BOD5 and COD values were 985 mg/L and 1556 mg/L, respectively. After treatment with immobilized and free cells, these values decreased to 310 mg/L and 510 mg/L respectively; there was significant variation at $p<0.05$ among the treatments. The better performance recorded in immobilized cells could be attributed to the carriers' ability to impart cell potency, which results in high bioutilization effectiveness. This investigation confirmed the work of Enerjiofi *et al.* (2017) and (Sonune and Garode, 2015) who reported the remarkable decline in COD and BOD_5 in cassava wastewater and industrial wastewater treated with bacterial mixed culture. Several other authors also reported the reduction in BOD_5 and COD during bioremediation of wastewater (Gaikwad *et al*., 2014).

The TSS of the CME was 2751 mg/l before treatment, but subsequently decreased to 990 mg/L and 710 mg/L respectively when treated with planktonic cells and immobilized cells during the 12-day bioremediation process. There is no significant variation in both treatment except in control samples at $p<0.05$. Similarly, the initial value of Total Dissolve Solid was (3470 mg/L) prior to bioremediation, which was remarkably reduced to (1550 mg/L) when treated with free cells, while (1100 mg/L) was recorded when treated with the immobilized cells. This trend vis-à-vis the total dissolve solid corroborated with the report of (Okoduwa *et al*., 2017; Izah *et al*., 2017) who reported reduction in total dissolve solid in Tannery effluent and cassava mill effluent respectively during bioremediation.

The initial value of phosphate recorded was 106 mg/L prior to bioremediation process, which later declined to 56 mg/L and 32 mg/L, when treated with planktonic cells and

immobilized cells respectively. A statistically significant difference was observed in both treatments at a significance level of $p<0.05$. This study agrees the work of Ajao *et al*. (2011) who reported remarkable reduction in phosphate content of textile effluent treated with immobilized *Bacillus* sp and *Pseudomonas* spp. The final phosphate concentration obtained after bioremediation process was within the permissible limit of FEPA.

The initial cyanide concentration in the cassava mill effluent used in this study was 25 mg/l before the bioremediation process. After 12 days of treatment, it decreased to 10 mg/L when free cells were used and to 4 mg/L when immobilized cells were employed. There was a significant difference between the two treatments ($p>0.05$). The initial nitrate concentration was 302 mg/L, and it reduced to 122 mg/L when treated with free cells and 96 mg/L with immobilized cells after the 12-day treatment. Significant variation was observed among the treatments ($p>0.05$).

This reduction in nitrate is consistent with the findings of Sonune and Garode (2015), who demonstrated the ability of *B. licheniformis* to reduce nitrate in municipal wastewater ($p>0.05$).

The cyanide reduction in this study agrees with the work of Nwokoro and Dibua, (2014) who reported remarkable reduction in cyanide in soil contaminated with cassava effluent using *P. stutzeri* and *B. subtilis* (Sankararayana and Gowthami, 2015) reported cyanide degradation up to 98 % using microbial consortia.

Incidentally, Ahmed *et al*. (2005) and Gupta *et al*. (2017) reported that the bacterial cells evolve different mechanisms in the presence of high levels of heavy metals in their environment, which could help to eliminate heavy metals from polluted sites. The heavy metals detected in the cassava mill effluent prior to bioremediation were Pb^{2+}, Ni^{2+}, Cr^{6+}, and Cu^{2+} with the initial mean values of 0.54 mg/l, 2.89 mg/l, 0.18 mg/l and 12.39 mg/l respectively. The concentrations were declined to 0.22 mg/l, 1.40 mg/l, 0.15mg/l and 8.58 mg/l when treated with free cells, while the following results were obtained when treated with the immobilized cells (0.06 mg/l), (0.02mg/l), (0.00mg/l) and (3.24 mg/l) and the values were below the permissible limit (Abo-Amer *et al.*, 2015), In their recent study, it was confirmed that *Alcaligenes faecalis* isolated from wastewater has a co-resistance capability to Cd^{2+}, Pb^{2+}, Ag^{2+} and Al^{3+}.

Mechanisms of metal removal capability of the microbes have been proposed to include the following: Redox transformations, metal-binding peptide and protein production, solubilization through secretion of siderophores and other complexing agents. Cell walls and other structural components have substantial metal-binding capabilities, while precipitation may result from metabolite release such as sulfide, oxalate or reduction (Gadd, 2004).

The remarkable outcomes of this study are in line with most of the published articles regarding the bioremediation of CME by several authors (Enerjiofi *et al.*, 2017; Izah *et al*., 2018) though this is the first report on the bioremediation of CME using immobilized cyanide degrading bacterial cells for the removal of physicochemical parameters and heavy metals with unprecedented findings.

5. Conclusion

Despite the high levels of physicochemical parameters and heavy metal concentrations in the CME, the immobilized and free cells showed a remarkable reduction in physicochemical parameters and heavy metal concentrations, indicating a promising approach for utilizing biological treatments to mitigate the negative consequences of CME on the environment and human health. Further research and application of this technique could lead to significant progress in managing cassava mill effluent in Nigeria and other regions facing similar challenges.

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