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Bioremediation of Cassava, Cocoa, and Palm Oil Industrial Effluents Using Indigenous Bacteria and Fungi

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Abstract

Release of untreated or partially treated industrial effluents into aquatic systems may pose significant risks to human, animal, and environmental health. This study investigated the bioremediation potentials of indigenous bacteria and fungi in some agro-based industrial effluents. Effluent samples (n=90) from cassava, cocoa, and palm oil industries in Ondo State, Nigeria were collected for five weeks. Bacteria and fungi in the effluent samples were isolated and identified using standard microbiological methods. The physicochemical characteristics of the effluent samples were determined using standard methods. Bacterial and fungal isolates with a high rate of utilization of the effluents were selected for the bioremediation assay. Results revealed that effluent samples from the palm oil industry had the highest bacterial and fungal counts, while effluents from the cocoa industry had the least. In all the effluent samples, *Bacillus*, *Lactobacillus,* and *Pseudomonas* were the most prevalent bacteria, while *Aspergillus* was the most prevalent fungi. The mean values of pH in cassava, cocoa, and palm oil effluent samples were 5.4, 6.4, and 4.4, respectively. High levels of cyanide and oil were detected in cassava and palm oil effluent samples, respectively. In terms of reduction of biological oxygen demand (BOD) and chemical oxygen demand (COD) in all the effluent samples, treatments containing the consortium of the isolates exhibited the highest bioremediation potential followed by treatments containing *Corynebacterium manihot* in cassava effluents; *Lactobacillus delbrueckii* in cocoa effluents; and *Penicillium notatum* in palm oil effluents. The findings of the study suggest that a consortium of the isolates, *C. manihot, L. delbrueckii,* and *P. notatum* represent promising microorganisms for bioremediation of cassava, cocoa, and palm oil industrial effluents.

Keywords: *bioremediation, environmental protection, agro-allied industrial effluents, microorganisms, pollution.*

1. Introduction

The threat to human and aquatic lives posed by industrial liquid and gaseous effluents cannot be over-emphasized, because industrial effluents are major sources of toxic contaminants in any environment (Kanu and Achi, 2011). Rapid industrialization and urbanization have enhanced the level of organic contaminants in the environment (Ethan *et al.,* 2003). Rivers and streams are constantly exposed to health-related risks as a result of indiscriminate discharge of untreated or partially treated industrial effluents (Adekunle and Eniola, 2008; Dash *et al.,* 2009; Kanu and Achi, 2011). The increased importance of cassava in agricultural and economic development as well as in food security particularly in Nigeria should give its processing and waste handling more attention (Arimoro *et al.,* 2008). During processing, three major waste streams are generated, these are liquid (cassava mill effluents), solid (cassava peels and sieves), and gaseous emissions. Cassava mill effluents are produced from various stages such as washing, grating, and moisture extraction processes. The quality of cassava mill effluents is assessed based on their physical, chemical, and biological constituents. Studies have shown that the physical and chemical constituents of cassava mill effluents released from different processing facilities often contaminate soils at the point of discharge i.e., the organic components in the effluents may negatively impact the soil ecosystem (Ogboghodo *et al*., 2006; Igbinosa, 2015).

The total installed cocoa processing capacity of Nigerian firms is at least 105,000 tons per annum while at least 45,000 tons is processed into intermediate products for local beverage industries or export. In Ondo State, one of the major tree crops is cocoa, its production and processing have earned the region an economic boost in recent years. Effluents from cocoa processing factories have been demonstrated to contain microorganisms and heavy metals that may pose a significant risk when discharged into the environment (Akinnusotu and Arawande, 2016). In addition, the palm oil industry has been growing rapidly as a result of the rising global demand for fats and oil, thus becoming a major contributor to the economy of several tropical countries, including Nigeria. Palm oil mill effluent (POME) is a brown slurry of organic solids (4-5%), residual oil (0.5-1.0%), and water (95%) which is generated during the multiple processing steps of crude oil production (Wu *et al*., 2009). POME has high organic content, biological oxygen demand (BOD), and chemical oxygen demand (COD) and is known to cause environmental problems such as eutrophication and water pollution (Osunbitan *et al.,* 2000; Kanu and Achi, 2011).

Generally, these effluents from cassava, cocoa, and palm oil processing factories contain substances that may be

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lethal, mobile in soil, affect biodiversity, cause the extinction of benthic macroinvertebrates and other aquatic lives, inhibit germination of cereal seed and alter microbial communities (Arimoro *et al.,* 2008; Wu *et al*., 2009; Akinnusotu and Arawande, 2016). It is important to subject these effluents to various treatment processes before discharging them into the environment (Shah, 2017). Bioremediation is a process in which beneficial microorganisms such as yeast, fungi, or bacteria are used to clean up contaminated soil or water. It is defined as the elimination, attenuation, or transformation of polluting or contaminating substances through the application of biological processes. It provides a technique for cleaning up pollution by enhancing the natural biodegradation processes (Enerijiofi *et al*., 2017; Ganapathy *et al*., 2019).

This study was aimed at determining the bioremediation potentials of indigenous bacteria and fungi in some agro-based industrial effluents. The specific objectives of this study were to isolate and identify bacteria and fungi from cassava, palm oil, and cocoa industrial effluents; assess the rate of utilization of the effluents by the isolates; determine the physicochemical characteristics of the effluent samples, and evaluate the bioremediation potentials of selected isolates on the effluent samples.

2. Materials and Methods

2.1 Description of the study area

Cassava effluents were collected from Matna Foods Company Limited, Akure – Owo expressway. Cocoa effluents were collected from Plantation Nigeria Limited, Akure and palm oil effluents were collected from Tohkl Palm Oil Mill, Aule Village, Akure. The agro-based industries are all located in Ondo State, Nigeria (Figure 1).

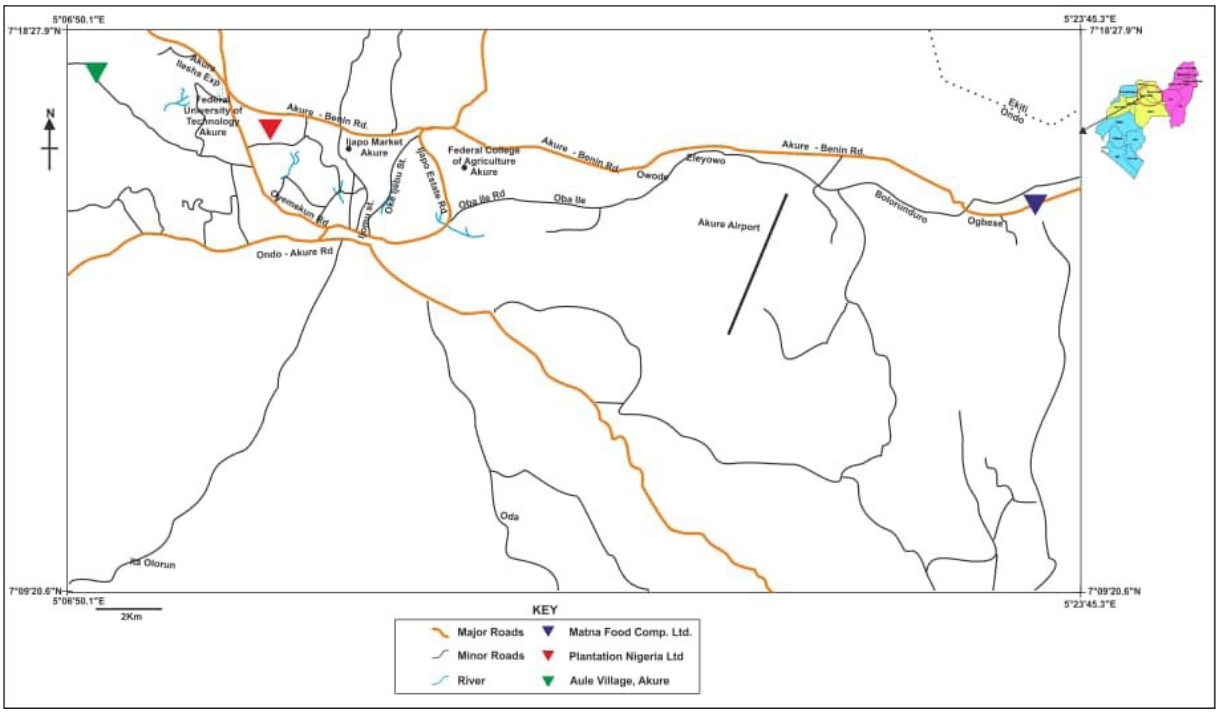

Figure 1. Map showing the sampling points of cassava, cocoa, and palm oil effluents.

2.2 Collection of effluent samples

Cassava, cocoa, and palm oil effluent samples were collected weekly over five (5) weeks to monitor trends and variations in microbial and physicochemical properties of the effluents. On each sampling occasion, effluents (1 L) were collected in duplicates at 10 am from their respective processing factories into sterile screw-capped bottles from each sampling point. The samples were stored in a cool box with ice packs and were transported to the laboratory within one (1) hour.

2.3 Enumeration and identification of bacterial and fungal populations in effluent samples

Serial dilutions were carried out weekly on each of the effluent samples and were cultured using the pour plate method. Nutrient agar (NA), Manitol Salt agar (MSA), Eosin Methylene Blue agar (EMB), De Manrogosa agar (MRS), Potato Dextrose agar (PDA), and mineral salt medium supplemented with 1% v/v of effluent samples were used for the enumeration of bacteria and fungi in the effluent samples. The agar plates were incubated at 35°C for 24 hours (NA, EMB, MSA, and MRS); and 25°C for 48 - 72 hours (PDA). Discrete colonies of bacteria and fungi were counted and expressed as colony-forming units per milliliter (CFU/ml) and spore-forming unit per milliliter (SFU/ml) respectively. The isolates were sub-cultured repeatedly to obtain pure isolates and were characterized further using morphological, physiological, and biochemical properties that included Gram reaction, catalase test, motility test, oxygen relation, sulphide test, indole production test, and carbohydrates (glucose, lactose, fructose, dextrose, maltose, tryptose, and sucrose) utilization test. Thereafter, the bacterial isolates were identified using Bergey's Manual of Determinative Bacteriology, and fungal isolates were identified using Practical Mycology: Manual for Identification of Fungi.

2.4 Determination of the rate of utilization of effluent samples by the isolates

The method of Enerijiofi *et al.* (2017) was adopted. The mineral salt medium (MSM) (oil agar medium) for palm oil utilizing bacteria was prepared to contain; 4 g NH_4Cl, 1.8 g K_2HPO_4, 1.2 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.01 g $FeSO_4$, 15 g agar and 1000 ml distilled water. For fungi, the medium had 10 g NaCl, 0.42 g $MgSO_4.7H_2O$, 0.29 g KCl, 0.83 g KH_2PO_4, 1.25 g Na_2HPO_4, 0.42 g $NaNO_3$, 20 g agar, 1000 ml distilled water and pH of 7.2. The medium was supplemented with 1% v/v of effluent as a carbon and nitrogen source. One milliliter of the serially diluted effluent sample was pure-plated on the media. Agar plates for bacteria and fungi were incubated appropriately and colonies were counted and recorded.

2.5 Determination of physicochemical characteristics of effluent samples

The temperature of the effluent samples was determined on-site using a mercury-in-glass thermometer. Electrical conductivity was determined using a conductivity meter (YSI Model 34). The pH, dissolved oxygen (DO), total dissolved solids, biological oxygen demand (BOD), chemical oxygen demand (COD), oil, cyanide, and total carbohydrate and ash content in the effluent samples were measured weekly using standard methods.

2.6 Determination of bioremediation potential of isolates on effluent samples

Shake Flask Degradation test as described by Enerijiofi *et al*. (2017) was adopted. An aliquot of 0.2 ml (standardized to 10^6 CFU/ml for bacteria and SFU/ml for fungi) of the inoculum of bacteria and fungi was dispensed into the flask containing 200 ml of sterilized cassava, palm oil, and cocoa mill effluents, separately in each treatment and mixed to form a consortium. All flasks were incubated at room temperature (28±2°C) on a rotary shaker at 120 rpm for 5 days. During the incubation period, temperature, pH, COD, BOD, and bacterial and fungal load were monitored at 24 hours intervals for five days.

2.7 Statistical analysis

Data obtained from this study were subjected to descriptive statistics. One-way Analysis of Variance was carried out and means were separated by Duncan's New Multiple Range test using SPSS version 22, at $P \leq 0.05$ level of significance. Values were presented as mean values ± standard deviation.

3. Results

3.1 Detection of bacteria and fungi in effluent samples

The total viable count of bacteria in cassava effluent samples ranged from 7.6×10^2 to 1.13×10^3 CFU/ml; 3.3×10^2 to 5.3×10^2 CFU/ml in cocoa effluent samples and 7.4×10^3 to 8.5×10^3 CFU/ml in palm oil effluent samples (Figure 2).

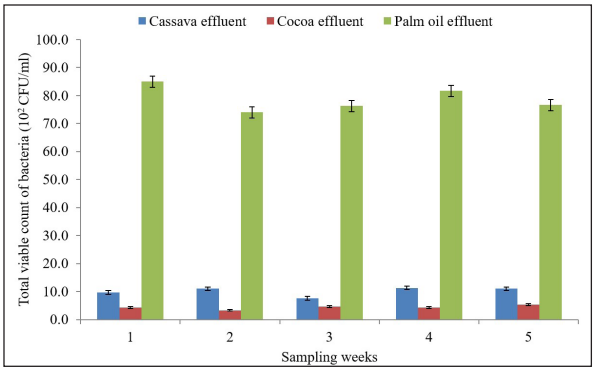

Figure 2. Total viable count of bacteria in effluent samples.

The total count of fungi in cassava effluent samples ranged from 2.3×10^3 to 5.3×10^3 SFU/ml; 2.0×10^3 to 4.6×10^3 SFU/ml in cocoa effluent samples and 9.0×10^3 to 1.06×10^4 SFU/ml in palm oil effluent samples (Figure 3).

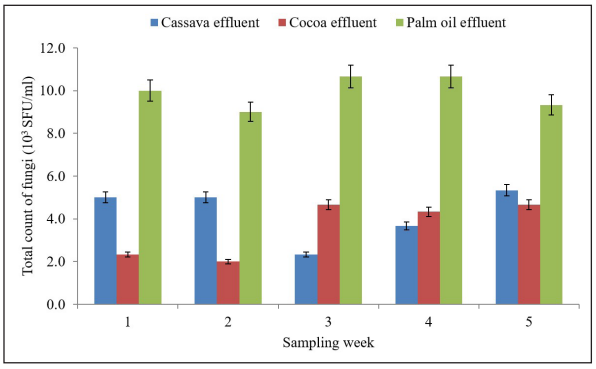

Figure 3. Total count of fungi in effluent samples.

Based on morphological and biochemical characteristics, the bacterial isolates detected in cassava effluent samples were *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Corynebacterium manihot*, *Morganella morganii*, *Lactobacillus acidophilus,* and *Lactobacillus fermentum*. In cocoa effluent samples, *L. delbrueckii*, *P. fluorescence*, *Proteus mirabilis,* and *B. subtilis* were isolated, whereas, in palm oil effluent samples, *S. aureus*, *B. cereus*, *M. luteus*, *Klebsiella pneumonia*, *Providencia vermicola*, *L. delbrueckii,* and *P. aeruginosa* were isolated. Of all the isolates, *Bacillus*, *Lactobacillus* and *Pseudomonas* were the most prevalent bacteria in cassava, cocoa, and palm oil effluent samples (Table 1).

The fungal isolates detected in cassava effluent samples were *Rhizopus stolonifer, Aspergillus fumigatus, A. niger, Saccharomyces cerevisiae,* and *Candida tropicalis.* In cocoa effluent samples, *S. cerevisiae, Penicillium notatum, A. fumigatus,* and *C. pelliculosa* were isolated, whereas, in palm oil effluent samples, *A. fumigatus, A. niger,* and *P. notatum* were isolated. Of all the isolates, *Aspergillus* species were the most prevalent fungi in cassava, cocoa, and palm oil effluent samples (Table 2).

Table 1. Frequency of occurrence of bacterial isolates in the effluent samples.

Effluent	Bacteria	Weeks					Occurrence (%)
		1	2	3	4	5	
Cassava	P. aeruginosa	+	+	+	-	-	13.0
	M. luteus	+	+	-	-	-	8.7
	B. subtilis	+	-	+	-	-	8.7
	S. aureus	-	+	-	+	-	8.7
	C. manihot	-	-	-	+	+	8.7
	M. morganii	-	+	+	+	+	17.4
	L. acidophilus	-	+	+	+	+	17.4
	L. fermentum	-	+	+	+	+	17.4
Cocoa	L. delbrueckii	+	+	+	-	-	27.3
	P. fluorescence	+	+	-	-	-	18.2
	P. mirabilis	-	+	+	+	+	36.3
	B. subtilis	-	-	-	+	+	18.2
Palm oil	S. aureus	+	+	-	-	+	18.8
	B. cereus	+	+	-	-	-	12.5
	M. luteus	-	-		+	+	12.5
	K. pneumoniae	-	-	+	+	+	18.8
	P. vermicola	-	+	-	-	-	6.2
	L. delbrueckii	-	+	-	+	+	18.8
	P. aeruginosa	+	-	+	-	-	12.5

Key: + = Present, - = Absent; Percentage of occurrence of each isolate was calculated by dividing the number of times an isolate was detected in the effluent samples by the total number of times all isolates were detected in the effluent samples over the five weeks multiplied by 100.

Table 2. Frequency of occurrence of fungal isolates in the effluent samples

Effluent	Fungi	Weeks					Occurrence (%)
		1	2	3	4	5	
Cassava	R. stolonifer	-	-	-	+	+	11.8
	A. fumigatus	+	-	+	-	+	17.7
	A. niger	-	+	+	+	+	23.5
	S. cerevisiae	-	+	+	+	+	23.5
	C. tropicalis	-	+	+	+	+	23.5
Cocoa	S. cerevisiae	+	+	+	-	-	27.3
	A. fumigatus	+	+	-	-	-	18.2
	P. notatum	-	+	+	+	+	36.3
	C. pelliculosa	-	-	-	+	+	18.2
Palm oil	A. fumigatus	+	+	-	-	+	42.9
	A. niger	-	+	-	-	-	14.3
	P. notatum	-	+	-	+	+	42.8

Key: + = Present, - = Absent; Percentage of occurrence of each isolate was calculated by dividing the number of times an isolate was detected in the effluent samples by the total number of times all isolates were detected in the effluent samples over the five weeks multiplied by 100.

3.2 Rate of the utilization of effluent samples by the isolates

The total count of bacteria and fungi on mineral salt medium (MSM) supplemented with the cassava, cocoa, and palm oil effluent samples were lower than the mean load of bacteria and fungi from the effluent samples cultured on general-purpose media (Table 3). *M. morganii*, *C. manihot*, *P. aeruginosa, C. tropicalis,* and *S. cerevisiae* demonstrated higher rates of utilization of cassava effluents. *P. fluorescence, L. delbrueckii, C. pelliculosa,* and *S. cerevisiae* showed higher rates of utilization of cocoa effluents, whereas *P. aeruginosa, M. luteus, P. vermicola* and *P. notatum* exhibited higher rates of utilization of palm oil effluents.

Table 3. Total count of bacteria and fungi on mineral salt medium and general-purpose media.

Effluent samples	Bacteria on MSM (CFU/ml × 10^4)	Bacteria on NA (CFU/ml × 10^6)	Fungi on MSM (SFU/ml × 10^2)	Fungi on PDA (SFU/ml × 10^3)
Cassava	2.57 ± 0.8^a	1.02 ± 1.3^b	3.7 ± 0.2^b	4.3 ± 0.6^b
Cocoa	2.07 ± 0.0^b	0.44 ± 0.7^c	4.3 ± 0.1^b	3.6 ± 0.6^c
Palm oil	2.24 ± 0.4^b	7.84 ± 1.6^a	6.2 ± 0.3^a	9.9 ± 0.4^a

Key: NA = nutrient agar, PDA = potato dextrose agar, CFU = colony forming unit, SFU = spore forming unit, ml = milliliter, MSM = mineral salt medium. Data presented as Mean ± SD, values in the same column with the same superscript are not significantly different ($p \leq 0.05$)

3.3 Physicochemical characteristics of effluent samples

The mean values of pH in cassava, cocoa, and palm oil effluent samples were 5.4, 6.4, and 4.4, respectively. The mean values of temperature of cassava, cocoa, and palm oil effluent samples were 30.2, 28.9, and 29.4 °C, respectively. The mean values of electrical conductivity in cassava, cocoa, and palm oil effluent samples were 1794.2, 473.1, and 572.2 µs/cm, respectively. Similarly, the mean values of total dissolved solids in cassava, cocoa, and palm oil effluent samples were 4635.1, 327.9, and 3728.3 mg/ml, respectively. The mean values of biochemical oxygen demand and chemical oxygen demand in cocoa effluent samples were lower than those observed in cassava and palm oil effluent samples. In addition, the mean values of total carbohydrate and ash content in palm oil effluent samples were higher than those observed in cassava and cocoa effluent samples. High levels of cyanide and oil were detected in cassava and palm oil effluent samples, respectively (Table 4).

Table 4. Physicochemical characteristics of effluent samples.

Parameters	Cassava effluent	Cocoa effluent	Palm oil effluent	FEPA Guideline
pH	5.4 ± 0.3^b	6.4 ± 0.5^a	4.4 ± 0.2^c	6-9
Conductivity (µs/cm)	1794.2 ± 0.9^a	473.1 ± 0.7^c	572.2 ± 0.6^b	750.5
Temperature (°C)	30.2 ± 0.2^a	28.9 ± 1.4^a	29.4 ± 0.2^a	25-28
Biochemical oxygen demand (mg/l)	1236.1 ± 12.1^b	22.2 ± 0.5^c	4362.0 ± 0.5^a	10
Chemical oxygen demand (mg/l)	1694.5 ± 14.0^b	98.7 ± 0.9^c	8615.1 ± 11.4^a	40
Total dissolved solids (mg/l)	4635.1 ± 1.4^b	327.9 ± 2.3^c	3728.3 ± 2.6^a	2000
Oil (mg/l)	ND	ND	9620.1 ± 3.4	10
Total carbohydrate (mg/l)	973.5 ± 3.0^b	629.1 ± 4.4^c	18443.1 ± 2.4^a	
Ash content (mg/l)	726.3 ± 0.5^b	6.8 ± 0.7^c	3928.1 ± 1.9^a	
Cyanide (mg/l)	21.0 ± 1.2	ND	ND	0.2

Key: ND = Non-detect; FEPA – Federal Environmental Protection Agency (1991); Data presented as Mean ± SD (n = 5), values in the same row with the same superscript are not significantly different ($p \leq 0.05$)

3.4 Bioremediation potential of isolates on cassava, cocoa, and palm oil effluents

In the bioremediation process of cassava effluents, the counts of bacteria, fungi, and their consortium in the treatments increased significantly ($p < 0.05$). The highest cell count was observed in the consortium (2.27×10^6 CFU/ml) followed by treatments containing *C. manihot* (1.59×10^6 CFU/ml) and *P. aeruginosa* (1.27×10^6 CFU/ml) (Table 5). As the bioremediation process progressed, the values of pH decreased steadily in all the treatments except the control, from slightly acidic to more acidic. In terms of the reduction of COD and BOD in the cassava effluents, the consortium exhibited the highest potential (COD – 39%; BOD – 35%) followed by treatments containing *C. manihot* and *P. aeruginosa* (Figure 4). Treatments containing bacterial isolates demonstrated higher bioremediation potential of cassava effluents compared to those containing fungal isolates.

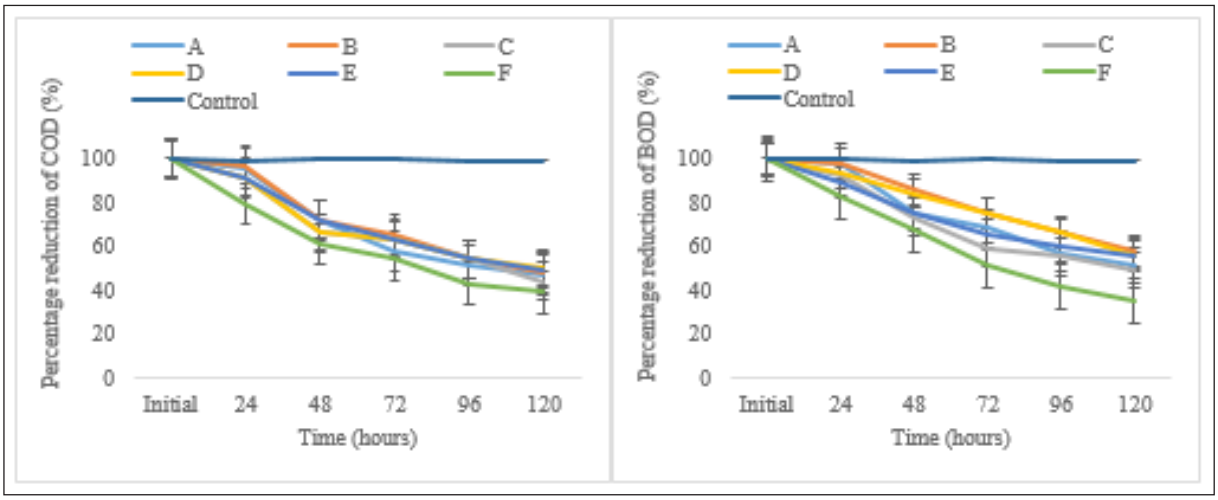

Figure 4. Percentage reduction of COD and BOD in cassava effluents over time (Treatments: A – *P. aeruginosa*; B – *M. morganii*; C – *C. manihot*; D – *S. cerevisiae*; E – *C. tropicalis*; F – consortium).

Table 5. Bioremediation potential of isolates on cassava effluents.

Treatments	Parameters	Time (h)					
		Initial	24	48	72	96	120
A	Count (×10⁴ CFU/ml)	-	11	31	53	92	127
	pH	5.4	5.3	5.1	4.9	4.7	4.3
	Temperature (°C)	30.2	32.2	33.5	33.2	33.6	33.8
B	Count (×10⁴ CFU/ml)	-	12	22	39	61	82
	pH	5.4	5.2	4.8	4.8	4.7	4.5
	Temperature (°C)	30.2	32	33.0	33.6	33.2	33.2
C	Count (×10⁴ CFU/ml)	-	15	48	67	104	159
	pH	5.4	5.2	5.0	4.3	4.3	3.9
	Temperature (°C)	30.2	32.1	34.4	34.5	34.8	34.9
D	Count (×10⁴ SFU/ml)	-	8	20	28	43	67
	pH	5.4	5.0	4.7	4.4	4.2	4.0
	Temperature (°C)	30.2	33.3	33.5	33.6	33.6	33.8
E	Count (×10⁴ SFU/ml)	-	8	21	37	51	72
	pH	5.4	5.0	4.8	4.5	4.1	3.9
	Temperature (°C)	30.2	32.8	33.0	32.8	33.2	33.2
F	Count (×10⁴ CFU/ml)	-	31	79	133	176	227
	pH	5.4	5.0	4.8	4.31	4.26	3.9
	Temperature (°C)	30.2	33.2	34.4	35.0	36.1	36.7
Control	Count	-	-	-	-	-	-
	pH	5.4	5.4	5.4	5.4	5.4	5.4
	Temperature (°C)	30.2	30.2	29.9	30	29.8	29.7

Key: Treatments: A – *P. aeruginosa*; B – *M. morganii*; C – *C. manihot*; D – *S. cerevisiae*; E – *C. tropicalis*; F – consortium

In the bioremediation process of cocoa effluents, the counts of bacteria, fungi, and their consortium in the treatments also increased significantly ($p < 0.05$). The highest cell count was observed in the consortium (1.43×10^6 CFU/ml) followed by treatments containing *P. fluorescence* (9.8×10^5 CFU/ml) and *L. delbrueckii* (8.4×10^5 CFU/ml). As the bioremediation process progressed, the values of pH decreased steadily in all the treatments except the control, from near neutral to slightly acidic (Table 6). In terms of the reduction of COD and BOD in the cocoa effluents, the consortium exhibited the highest potential (COD – 32%; BOD – 65%) followed by treatments containing *L. delbrueckii* and *P. fluorescence* (Figure 5). Similarly, treatments containing bacterial isolates demonstrated higher bioremediation potential of cocoa effluents compared to those containing fungal isolates.

Table 6. Bioremediation potential of isolates on cocoa effluents.

Treatments	Parameters	Time (h)					
		Initial	24	48	72	96	120
A	Count (×10⁴ SFU/ml)	3	7	17	24	37	46
	pH	6.4	6.4	5.9	5.6	5.1	4.8
	Temperature (°C)	29	29	30	29	29	29
B	Count (×10⁴ SFU/ml)	3	4	9	13	17	22
	pH	6.4	6.2	5.6	4.7	4.6	4.5
	Temperature (°C)	29	29	30	29.7	29.8	29.7
C	Count (×10⁴ CFU/ml)	3	13	23	46	59	84
	pH	6.4	6.4	5.4	4.6	4.6	4.3
	Temperature (°C)	29	29	30	30	29.5	29.6
D	Count (×10⁴ CFU/ml)	3	12	27	49	71	98
	pH	6.4	6.4	5.8	4.8	4.8	4.5
	Temperature (°C)	29	29	30	29.7	29.8	29.9
E	Count (×10⁴ CFU/ml)	5	23	47	69	93	143
	pH	6.4	6.2	5.2	4.8	4.8	4.2
	Temperature (°C)	29	29	30	29.7	29.8	29.9
Control	Count	-	-	-	-	-	-
	pH	6.4	6.5	6.2	6.4	6.4	6.4
	Temperature (°C)	29	29	29	29	29	28.9

Key: Treatments: A – *S. cerevisiae*; B – *P. notatum*; C – *L. delbrueckii*; D – *P. fluorescence*; E – consortium

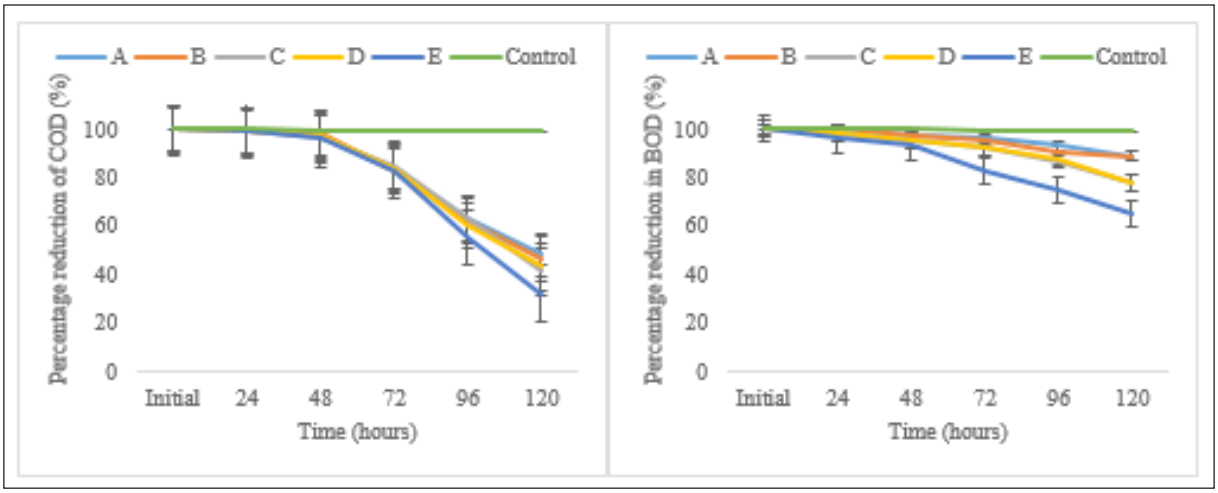

Figure 5. Percentage reduction of COD and BOD in cocoa effluents over time (Treatments: A – *S. cerevisiae*; B – *P. notatum*; C – *L. delbrueckii*; D – *P. fluorescence*; E – consortium).

In the bioremediation process of palm oil effluents, the counts of bacteria, fungi, and their consortium in the treatments increased significantly ($p < 0.05$). Again, the highest cell count was observed in the consortium (1.27×10^6 CFU/ml) followed by treatments containing *M. luteus* (8.8×10^5 CFU/ml) and *P. aeruginosa* (5.6×10^5 CFU/ml). As the bioremediation process progressed, the values of pH decreased slightly in all the treatments except the control (Table 7). In terms of the reduction of COD and BOD in the palm oil effluents, the consortium exhibited the highest potential (COD – 39%; BOD – 29%) followed by treatments containing *P. notatum* and *M. luteus* (Figure 6). Although the treatment containing *P. notatum* had the least count after 120 h, it demonstrated a higher bioremediation potential of palm oil effluents compared to those containing bacterial isolates.

Table 7. Bioremediation potential of isolates on palm oil effluents.

Treatments	Parameters	Time (h)					
		Initial	24	48	72	96	120
A	Count ($\times 10^4$ CFU/ml)	3	7	13	21	34	47
	pH	4.4	4.2	4.1	4.3	4.3	4.3
	Temperature (°C)	29.4	29.5	29.7	29.7	29.8	29.9
B	Count ($\times 10^4$ CFU/ml)	3	13	23	49	66	88
	pH	4.4	4.2	4.1	4.1	4.1	4.1
	Temperature (°C)	29.4	29.7	29.7	29.9	30.2	30.6
C	Count (CFU/ml)	3	9	13	25	37	56
	pH	4.4	4.4	4.4	4.3	4.3	4.2
	Temperature (°C)	29.4	29.5	29.5	29.6	29.7	29.8
D	Count (SFU/ml)	3	4	13	23	31	46
	pH	4.4	4.2	4.1	4.1	4.1	4.0
	Temperature (°C)	29.4	29.8	29.9	30.1	30.6	30.7
E	Count ($\times 10^4$ CFU/ml)	3	21	44	79	92	127
	pH	4.4	4.2	4.1	4.0	3.9	3.8
	Temperature (°C)	29.4	29.8	30.3	30.7	31.0	31.1
Control	Count	-	-	-	-	-	-
	pH	4.4	4.4	4.4	4.4	4.4	4.4
	Temperature (°C)	29.4	29.0	28.7	28.4	28.5	28.6

Key: Treatments: A – *P. vermicola*; B – *M. luteus*; C – *P. aeroginosa*; D – *P. notatum*; E – consortium

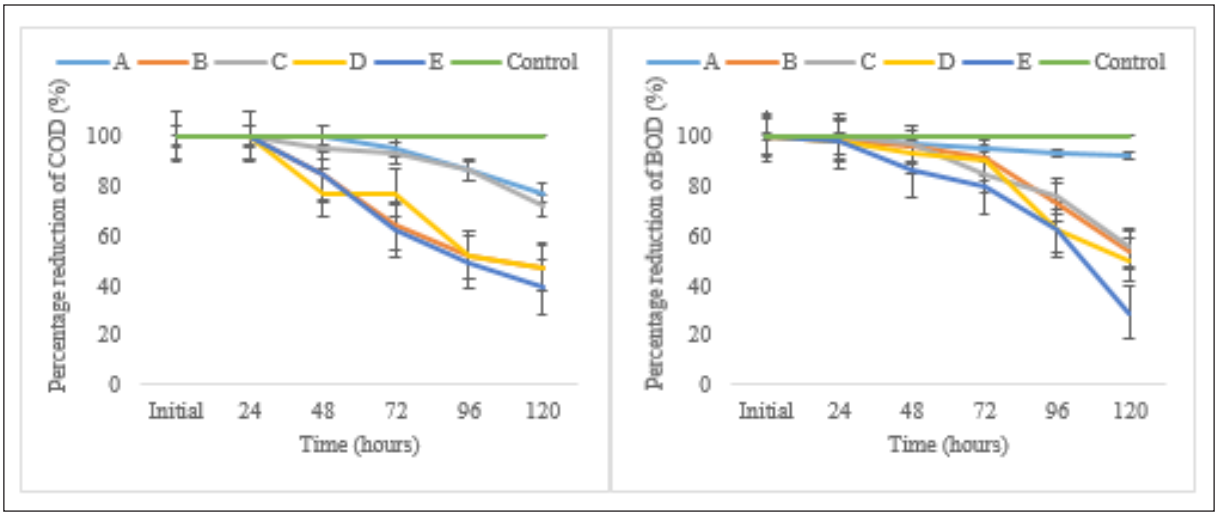

Figure 6. Percentage reduction of COD and BOD in palm oil effluents over time (Treatments: A – *P. vermicola*; B – *M. luteus*; C – *P. aeroginosa*; D – *P. notatum*; E – consortium).

4. Discussion

The bioremediation potentials of indigenous bacteria and fungi in some agro-based industrial effluents were examined. The total viable counts of bacteria and fungi that were higher in palm oil effluents compared to the counts in cassava and cocoa effluents may be a result of higher concentrations of carbohydrates, ash, and oil in the palm oil effluents. The presence of cyanide in cassava effluents may have also contributed to the observed lower counts of bacteria and fungi (Ogboghodo *et al*., 2006; Dash *et al*., 2009). The array and range of counts of bacteria and fungi observed in this study were similar to those reported by Akinnusotu and Arawande (2016) and Enerijiofi *et al.* (2017). The high prevalence of *Bacillus*, *Lactobacillus*, *Pseudomonas,* and *Aspergillus* observed in cassava, cocoa, and palm oil effluents may likely be because of the favorable pH and available nutrients in the effluents.

Enerijiofi *et al.* (2017) had earlier reported that growth media must be supplemented to isolate indigenous microorganisms from effluent. This may be responsible for the higher load of bacteria and fungi observed on mineral salt medium (MSM) supplemented with the cassava and cocoa effluents compared with their growth on general-purpose media. However, this trend was not observed on mineral salt medium (MSM) supplemented with palm oil effluents, as a load of bacteria and fungi was lower than those on general-purpose media. Again, this may be a result of higher concentrations of carbohydrates, ash, and oil in the palm oil effluents. Bacteria and fungi that demonstrated higher rates of utilization of cassava, cocoa, and palm oil effluents suggest that the isolates had the potential in utilizing effluents as their carbon and energy source (Enerijiofi *et al*., 2017; Shah, 2017; Ganapathy *et al*., 2019).

The pH of cocoa effluent was within the reference standard while the pH of cassava and palm oil effluents was lower than the standard value set by FEPA (1991) on National Guidelines and Standards for Industrial Effluents in Nigeria. The low pH of cassava effluent may be due to the high cyanide content while the low pH observed in palm oil may be a result of organic and free fatty acids arising from the partial degradation of palm fruits before processing. The electrical conductivity in cassava effluents that was above the recommended standard FEPA (1991) may be due to high levels of anions in cassava tubers (Enerijiof *et al*., 2017). The concentration of total dissolved solids in cassava and palm oil effluents was above FEPA standard (1991), this may be due to the contribution of inherent components of the product to the effluents during processing (Wu *et al*., 2009; Enerijiofi *et al.* 2017).

The temperature of all the effluents was higher than the standard limit, this could be a result of the temperature of the environment, especially in the tropics. The observed temperature is optimum for microbial growth and may be responsible for the array of bacteria and fungi isolated from the effluents (Akinnusotu and Aranwade, 2016; Shah, 2017). The levels of BOD and COD of all the effluents were above the standard limit, this may be attributed to the presence of high organic matter in the effluents (Okunade and Adekalu, 2013). The effluents may cause environmental problems such as eutrophication, water pollution loss of biodiversity, and soil fertility when discharged directly into the environment without treatment (Ogboghodo *et al*., 2006; Ezeigbo *et al*., 2014; Igbinosa, 2015). Bioremediation entails utilizing microorganisms to clean up contaminants in the environment and some of the microorganisms employed include members of the genera *Pseudomonas, Flavobacterium, Bacillus*, and *Serratia* (Enerijiofi *et al.,* 2017). The process is cost-effective and immobilizes contaminants in a manner that protects human health and the environment (Wu *et al.,* 2009; Bala *et al*., 2014). In the bioremediation process, the highest cell counts were observed in the treatments containing the consortium of indigenous microorganisms, this is in agreement with the report of Jameel and Olanrewaju (2011) where the authors observed that the right consortium is central to optimizing degradation activity. Counts of *C. manihot, P. aeruginosa,* and *M. morganii* were higher than other microorganisms in cassava effluents, counts of *P. fluorescence* and *L. delbrueckii* were higher than others in cocoa effluents, whereas, counts of *M. luteus* and *P. aeruginosa* were higher than others in palm oil effluents. These growth patterns

suggest that the isolates and consortium effectively utilized the effluent as nutrient or energy sources (Vijayaraghavan *et al*., 2007; Jameel and Olanrewaju, 2011; Enerijiofi *et al*., 2017).

The pH of all the effluents was reduced during the bioremediation process with indigenous microbial isolates and their consortium. This may be a result of the conversion of the organic compounds in the effluents to organic acids (Bala *et al.,* 2014; Jameel and Olanrewaju, 2011; Enerijiofi *et al*., 2017). Treatments containing the consortium of isolates exhibited the highest reduction in BOD and COD values in all the effluents. Improved pollutants remediation using bacterial and fungal consortiums had previously been reported (Jameel and Olanrewaju, 2011). In cassava effluents, treatments containing *C. manihot* and *P. aeruginosa* reduced the BOD and COD more effectively than other isolates. In cocoa effluents, *L. delbrueckii* and *P. fluorescence* reduced the BOD and COD more effectively than other isolates, whereas in palm oil effluents *P. notatum* and *M. luteus* were more effective than other isolates.

5. Conclusions

The findings of this study demonstrate the bioremediation potentials of *C. manihot, L. delbrueckii, and P. notatum* and their consortium on cassava, cocoa, and palm oil effluents, respectively. The mean percentage reduction of COD and BOD in the three effluents was approximately 37% and 43%, respectively. Increasing the concentration of the isolates in the treatments and improving the strains of the isolates may increase the efficiency of the bioremediation process. Treatment of agro-based industrial effluents with these promising isolates before discharge may offer an improved way of eliminating organic contaminants in the receiving soils, rivers, or streams, thereby protecting human and environmental health.

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