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Bio-treatment capabilities of powdered *Moringa oleifera* seeds and a consortium of some bacterial isolates in cassava mill effluent (CME)

[1] *Enerijiofi, K. E., [1]Addeh, A. O. [2]Ekhaise, F. O.

[1]Department of Biological Sciences, College of Basic and Applied Sciences, Glorious Vision University (formerly Samuel Adegboyega University), Ogwa, Edo State, Nigeria
[2]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

Abstract

Access to portable water supply is a serious challenge worldwide. Therefore, the development of sustainable, environmentally friendly wastewater management and recycling methods have become essential. This study evaluated the ability of powdered *Moringa oleifera* seeds and a consortium of *Bacillus* and *Pseudomonas* species to treat cassava mill effluent (CME) by monitoring the physicochemical parameters using standard methods. The bacterial counts revealed total coliform and total viable counts of 5±0.17 x 10^5 cfu/ml and 4.8±0.32 x 10^5 cfu/ml respectively. At the end of the 7-day bio-treatment of cassava mill effluent by *Moringa oleifera* seeds, the turbidity reduced by 79.45%. The total organic carbon had a reduction of 92.99% using *Moringa oleifera* seeds while the consortium gave 69.17%; the biochemical oxygen demand reduced by 99.30% using *Moringa oleifera* while the consortium was by 72.41%. Chemical oxygen demand had a reduction of 94.25% using *Moringa oleifera* seeds, while the consortium of bacterial isolates gave 83.34%. The pH reported was within the acidic range, while the temperature slightly decreased with increasing days of bio-treatment. Biodegradation indices showed a strong positive correlation between BOD and COD in both CME treatments. This study reports the efficacy of *Moringa* powdered seeds and a consortium of *Bacillus* and *Pseudomonas* species in the treatment of cassava mill effluent.

Keywords: Biodegradability index, environmental sustainability, microbial communities, *Moringa oleifera,* water quality

***Corresponding author:** kingsmore1luv@gmail.com; https://orcid.org/0000-0001-7513-4052

Introduction

Freshwater supplies are rapidly diminishing, leading to competition for scarce water resources. This is a foremost distress and an essential constituent of the United Nations Sustainable Development Goals (SDGs). It's been stated that the total volume of water that can be used on earth is only about 200,000 km hence the prominence of water quality, security, and management. With about 45 million metric tons of cassava produced yearly and Nigeria being the world's largest cassava producer, and its cassava industry is the most sophisticated. Cassava mill effluents (CME) are being produced from various stages involved in processing like washing, grating, and moisture extraction processes (Enerijiofi et al., 2017; Eze and Onyilide, 2015). The CME that arises from the outdated breakdown process throughout handing out is one of the main sources of environmental dilapidation, leading to pollution of agrarian farmlands, streams and upsetting the different forms of life (Izah et al., 2018).

The wastewater from cassava processing could serve as a substitute for mineral fertilizers, animal feeds, biofuel, and a portable water source if treated with microorganisms, thereby reducing contamination of the environment (Osadebe and Okounim, 2020). *Moringa oleifera* seed is a natural coagulant that is non-toxic, biodegradable, cost effective, available, low sludge volume, and eco-friendly for the treatment of CME compared to commercial coagulants such as calcium, aluminum, and their salts (Ibiene et al., 2021; Gautam and Saina*,* 2020; Verma et al., 2020). The significant use of *Moringa oleifera* seed extracts for wastewater treatment has been reported for effectively removing suspended material, softening hard waters, removing turbidity, COD, colour, and other organic pollutants (Adejumo et al., 2013).

Bio-treatment is the use of living organisms to clean up contaminants in the environment (Enerijiofi and Chukwuma, 2018; Enerijiofi and Ikhajiagbe, 2021). Some of the microorganisms employed include members of the gram-positive rod and some fungi, as reported by earlier researchers (Ajuzie et al., 2015; Eziegbo et al*.,* 2014). *Moringa oleifera* has also been used in the cleanup of contaminants. However, studies on the use of both *Moringa oleifera* and microorganisms in the bio-treatment of cassava mill effluent are lacking, making this an eye-opener for researchers to investigate. In Ogwa and Ebelle, like other cities in Nigeria, the processing of cassava is largely completed through self - help and the uncontrolled release of wastewater is a very common practice. The study was set out to treat cassava mill effluent using a screened indigenous consortium of *Bacillus* and *Pseudomonas* species as well as powdered seeds of *Moringa oleifera*.

Materials and Methods

Description of the Study Area

Ogwa and Ebelle are adjourning communities in Esan land but are located in Esan-West and Igueben Local Government Areas, respectively. The inhabitants of the area are predominantly farmers with cassava production and its products being their main farm produce. The study locations are normally moist, with the highest rainfall in the months of July and September. The annual mean rainfall is about 1,650 nm, with a mean annual temperature of 37.3^{o}C. The average mean relative humidity is 73.2% (NIMET, 2012).

Collection of cassava mill effluent and Moringa oleifera seeds.

Cassava mill effluent was collected with sterile plastic four (4) litre containers from cassava processing mills at Ogwa and Ebelle communities. The *Moringa oleifera* seeds were collected from a full-grown tree in a residential apartment at Ekewan area in Benin City, Edo State, Nigeria. The seeds were properly washed using sterile water to eliminate dust and peripheral constituents. The seeds were identified by a Professor of Botany, Mrs. Foluso May Ogbe. The samples were transported to the laboratory for further analyses.

Preparation of Moringa oleifera powdered seed-based coagulant.

The seeds were air dried and manually blended using a laboratory blender model MD-326S, China. The powdered samples were sieved to remove large particles and thereafter stored in well-labelled polymer bags. The prepared samples were left in the dark at room temperature until needed. The powder was mixed with sterile distilled water to attain the stock solution of 2% suspension. The suspension was agitated using a sterile glass rod to ensure uniform mixture. The solution was centrifuged at 120 rpm for 12 minutes to obtain the active ingredients in the powdered samples. Thereafter, Whatman's No. 1 filter paper was used in filtering the milky solution. The extract was used for evaluating the potential cassava mill effluent treatment (Ibiene et al., 2021).

Determination of physicochemical parameters

The physicochemical parameters that were assessed in the present study includes turbidity, total organic carbon (TOC), biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH, and temperature (APHA, 2011; Enerijiofi et al., 2017; Ibiene et al*.,* 2021).

BOD: CME sample was collected and divided into two portions. In the first portion, the dissolved oxygen was determined through the Wrinkler's method at the spot. The portion of the CME sample was used to fill an incubation bottle to the brim. The bottle was screw capped and incubated at 20^0C for 5 days. The difference between the first and second readings was recorded as the BOD value.

COD: The COD value for the CME sample was done by the colorimetric method. Ten milligrams per litre (10 mg/l) of the sample was measured and added to 5 ml of high range COD reagent (HACH). This mixture was positioned in a COD reactor for about 1 hour and allowed to cool. Thereafter, the mixture' absorbance was read at a wavelength of 346nm with the aid of HACH DR 2010 Spectrophotometer.

pH: The hydrogen ion concentration (pH) of the samples were measured with a HMDPHM 80 digital pH meter. Freshly prepared pH buffers of (7.00), (4.00) and (9.00) were used in calibrating the pH meter before and after each reading. The probe in the electrode was placed in about 20 ml of CME sample inside a glass beaker. The result displayed on the screen was read and thereafter recorded.

Turbidity: The turbidity values of the different CME samples were read with a spectrophotometer. Five millilitres of CME sample measured into a cuvette and put on the light chamber. The absorbance value was read at a specified wavelength in nepholometer turbidity unit (NTU) while distilled water was used as blank.

Temperature: This was determined using a thermometer. The thermometer was dipped into about 20 ml of CME sample inside a glass beaker. The result was read and thereafter recorded.

TOC: The colorimetric method was employed.

Determination of Total Heterotrophic Bacterial Count

One mililitre (1 ml) of cassava mill effluent was dispensed into 9 ml of distilled water in a test tube to give the stock solution. This was serially diluted in Ten - fold. Thereafter, aliquot of 1 ml appropriate dilutions (10^{-3} and 10^{-6}) of the cassava mill effluent sample was inoculated into Nutrient agar plates impregnated with fuscin and Eosin Methylene blue (EMB). The inoculated plates were incubated using an incubator at 35^{o}C for 48hrs to determine the total counts. The colony counter (Model- Labtech) was used to count visible discrete colonies on inoculated plates and expressed in colony forming units per millilitre (cfu/ml) (Enerijiofi et al., 2017).

Determination of Total Coliform Count

The multiple tube fermentation method was used in enumerating the total coliform count in a specified quantity of cassava mill effluent sample. (APHA, 2011). The cassava mill effluent was inoculated into lactose containing liquid medium and incubated at 37°C for 48 hours. The coliform bacteria were identified and counted by their capability to grow and release gas. The abundance of coliforms was detected by counting the number of positive and negative tubes and compared to the standard MPN table. Confirmatory tests were carried out for the presumptive positive tubes and to verify the presence of coliform as well as to detect any false positive results. Samples from the positive test tubes were inoculated on EMB medium at incubated for 48hours to confirm the presence of presence of coliforms.

Characterisation of Bacterial Isolates

Sub-culturing was used to separate the distinct colonies on freshly prepared Nutrient agar plates. Characterization and identification of pure cultures of bacterial isolates were based on their cultural, morphological, biochemical and sugar fermentation characteristics (Cheesbrough, 2006; Holt et al., 1994).

Bio-treatment of Cassava Mill Effluent (CME)

Each bacterial and fungal isolate of inoculum size 0.1 ml (10^6 spores/ml) was introduced into 500 ml Erlenmeyer flasks containing 100 ml each of raw CME. They were incubated at 30ºC on a rotary shaker (200 rpm). Samples were taken daily for 7 days to determine the changes in turbidity, total organic carbon (TOC), biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH, and temperature (Ibiene et al*.,* 2021).

Determination of bio-treatment efficiency

The efficiency of bio-treatment was calculated following the method of (Ibiene et al*.,* 2021).

$$\% \text{ efficiency} = \frac{\text{Initial parameter conc. in the raw CME} - \text{Final parameter conc. after treatment}}{\text{Initial parameter conc. in the raw CME}} \times 100\%$$

Determination of Correlation between COD and BOD and the Biodegradability Index (BI)

The biodegradability index measures the relationship between chemical oxygen demand and biochemical oxygen demand for wastewater samples. It is known as the ratio of BOD to COD at different points. The plots of BOD values against COD values were gotten and used in regression analysis to develop the corresponding correlation coefficients (Abdalla and Hammam, 2014).

Results

The mean total coliform and total viable counts were 5 ±0.17 x 10^5 cfu/ml and 4.8 ±0.32 x 10^5±0.32 cfu/ml, respectively (Table 1). Table 2 revealed the bacterial isolates identified and the frequency of occurrence (%) of the bacterial isolates (*Bacillus* and *Pseudomonas* species) with 50% each. Table 3 recorded the bio-treatment potentials of cassava mill effluent by *Moringa* seeds. Turbidity showed a percentage reduction value of 79.45%, total organic carbon was reduced by 92.99%, biological oxygen demand had a 99.30% reduction, chemical oxygen demand was 94.25%, while temperature gave 5.30% reduction. Table 4 recorded the bio-treatment potentials of cassava mill effluent by a consortium of *Bacillus* and *Pseudomonas* species. Total organic carbon showed a percentage reduction value of 69.17%, biological oxygen demand had 72.41% reduction, chemical oxygen demand gave a reduction of 83.34%, while temperature gave a reduction of 9.37%. The biodegradability indices based on the mean values for the biochemical oxygen demand and the chemical oxygen demand for the cassava mill effluent samples treated with powdered *Moringa oleifera* seeds was 0.4473 (Figure 1) while the consortium of *Bacillus* and *Pseudomonas* species gave 0.7506 (Figure 2).

Table 1: Total coliform counts and total viable bacterial counts

	TCC (cfu/ml)	TVBC (cfu/ml)
Cassava effluent	$5 \times 10^5 \pm 0.17$	$4.8 \times 10^5 \pm 0.32$

Legend: TCC – Total Coliform Count, TBC- Total Bacterial Count, cfu/ml - Colony Forming Unit per mililitre

Table 2: Percentage frequency of occurrence of bacterial isolates

ISOLATE	FREQUENCY	% OCCURRENCE
***Bacillus* sp.**	2	50
***Pseudomonas* sp.**	2	50
Total	4	100

Table 3: Bio-treatment of Cassava Effluent with *Moringa oleifera* Seed over a 7- day period

Parameters	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	& reductio
Turbidity (NTU)	73.0±0.02	41.0±0.32	26.0±0.10	21.0±0.23	18.0±0.42	18.0±0.31	16.0±0.16	15.0±0.52	79.45
TOC (%)	41.52±0.01	30.59±0.13	12.91±0.56	9.68±0.49	5.47±0.44	5.20±0.11	3.84±0.32	2.91±0.32	92.99
BOD(mg/l)	11.44±0.32	7.09±0.41	9.49±0.32	3.74±0.27	7.99±0.29	5.14±0.41	2.31±0.19	1.51±0.32	99.30
COD(mg/l)	592.0±0.04	198±0.11	64.08±0.87	106±0.38	74.51±0.30	53.36±0.52	50.25±0.20	34.02±0.32	94.25
pH	3.01±0.21	2.23±0.17	3.78±0.19	3.62±0.42	2.87±0.36	2.84±0.22	2.58±0.44	3.27±0.32	
Temperature	26.4±0.03	26.4±0.11	26.0±0.98	27.0±0.15	26.3±0.51	25.9±0.74	25.6±0.22	25.0±0.32	5.30

Legend: TOC = total organic carbon; BOD = biochemical oxygen demand, COD = chemical oxygen demand

Table 4: Bio-treatment of Cassava Effluent with a consortium of *Bacillus* and *Pseudomonas* species over a 7- day period

Parameters	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	% reduction
Turbidity NTU)	73.0±0.12	88.2±0.20	101.0±0.33	106.8±0.14	138.1±0.44	126.0±0.89	118.0±0.43	117.5±0.29	
TOC (%)	41.52±0.15	35.40±0.49	29.06±0.67	26.81±0.71	28.40±0.09	19.1±0.03	17.5±0.01	12.8±0.13	69.17
BOD (mg/l)	10.15±0.21	6.0±0.42	9.45±0.22	5.10±0.03	4.40±0.70	4.0±0.07	3.61±0.18	2.80±0.17	72.41
COD (mg/l)	592.0±0.41	420.8±0.18	318.1±0.06	229.8±0.50	142.5±0.10	120.2±0.02	102.0±0.25	98.6±0.32	83.34
Ph	3.04±0.17	2.65±0.76	3.66±0.61	3.50±0.29	3.15±0.03	3.01±0.11	2.87±0.13	3.25±0.20	
Temperature (^{0}C)	28.0±0.22	26.0±0.37	26.9±0.04	26.6±0.29	26.6±0.44	26.0±0.09	26.5±0.53	25.6±0.27	9.1w37

Legend: TOC = total organic carbon; BOD = biochemical oxygen demand, COD = chemical oxygen demand

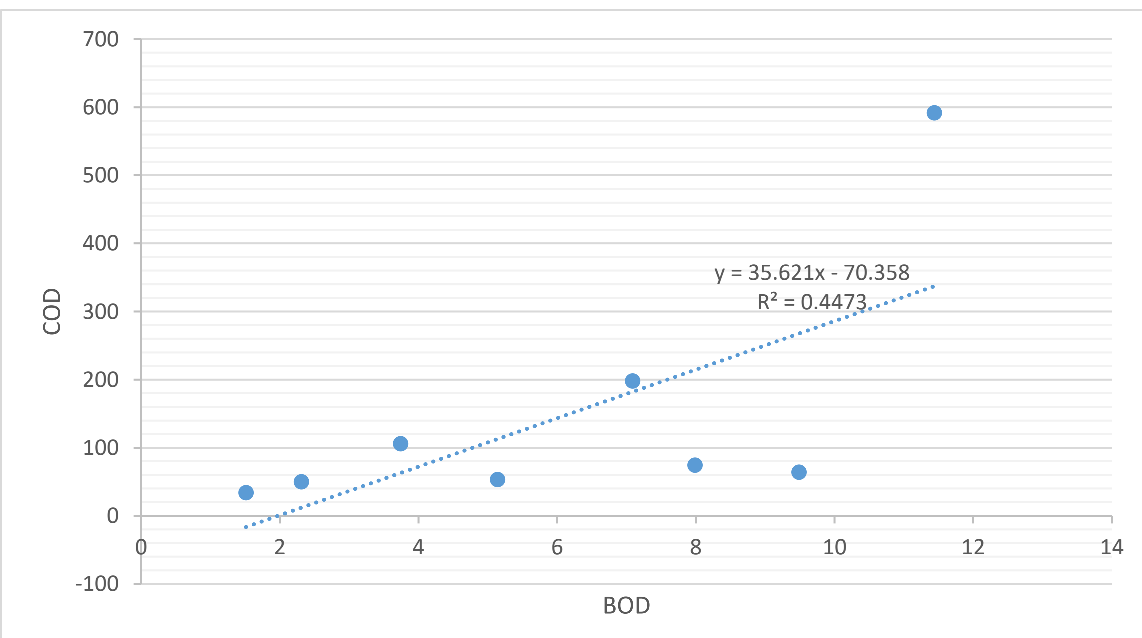

Figure 1: correlation between BOD and COD of Cassava Effluent with *Moringa oleifera* Seed

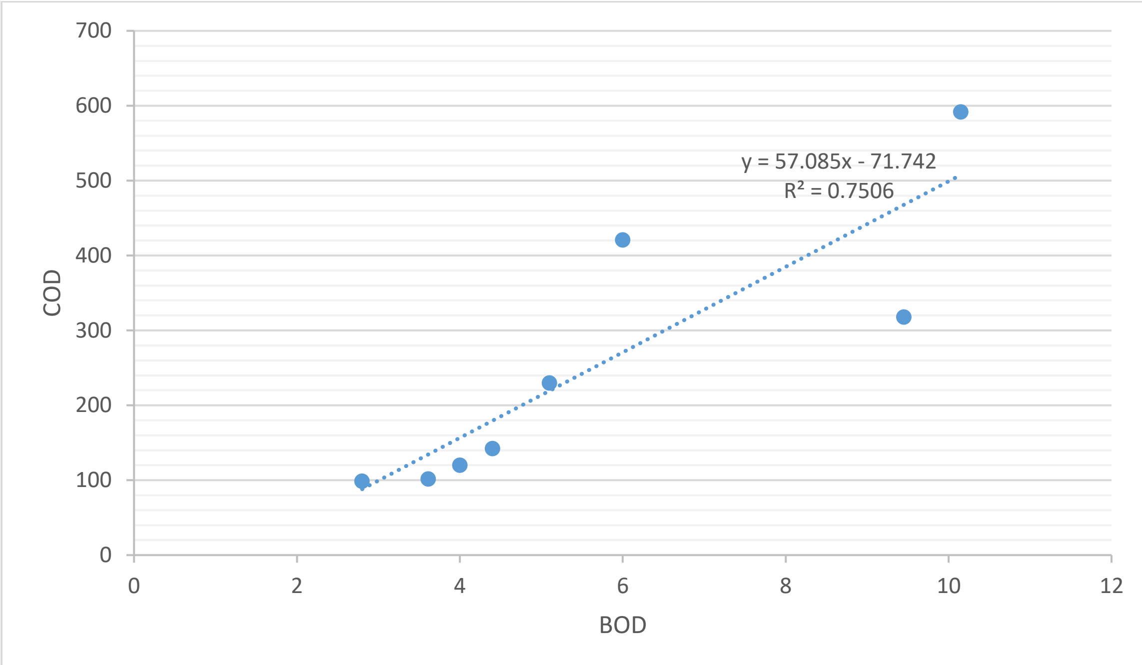

Figure 2: correlation between BOD and COD of Cassava Effluent with a consortium of *Bacillus* and *Pseudomonas* species

Discussion

The results showed mean total coliform and total viable counts of 5 ±0.17 x10^5 cfu/ml and 4.8 ±0.32 x10^5 cfu/ml respectively. However, Enerijiofi et al. (2017) reported a mean heterotrophic bacterial count of 3.4±0.26 x 10^6cfu/ml from cassava mill effluent (CME) samples. The counts revealed that cassava mill effluent was rich in nutrients that supported bacterial growth as recorded in this study. The presence of coliform counts in CME was an indication of contact with the soil environment and faeces of humans involved in the various stages of processing. Their presence also indicates that disease causing organisms could be present in the CME. The bacterial species that had profuse growth were *Bacillus* and *Pseudomonas* species, with a percentage frequency of occurrence of 50% each. However, the bacteria reported in this study are in consonance with earlier studies (Omotioma *et al*., 2015; Enerijiofi and Chukwuma, 2018). Also, Enerijiofi et al. (2017) isolated the genus *Pseudomonas* (15.48%), *Bacillus* (12.41%), *Acetobacter* and *Rhizopus* (10.88%), *Corynebacterium* (9.33%), *Lactobacillus*, *Micrococcus,* and *Aspergillus* (7.79%), *Staphylococcus* and *Penicillium* (6.25%), and *Saccharomyces* species (4.62%). These organisms, *Bacillus* and *Pseudomonas* species, are known to be eco-friendly biodegraders of environmental contaminants (Ikhajiagbe et al*.,* 2021).

Turbidity measures the cloudiness or thickness of any solution. The bio-treatment potentials of cassava mill effluent by *Moringa oleifera* seeds showed that turbidity was reduced by 79.45%. This agrees with the report of Hendrawati *et al*. (2015) that reported a 97.9% – 98.6% reduction in turbidity using 10% as the optimum concentration of powdered seed of *Moringa oleifera*. However, Ibiene et al. (2021) reported a 2% reduction. Madrona et al. (2012) suggested that active agents from the powdered seed of *Moringa oleifera* and the consortium of *Bacillus* and *Pseudomonas* species were able to reduce the turbidity by binding to the scums in the cassava mill effluent, leading to their removal. This result shows that there was a substantial improvement in the turbidity levels of the cassava mill effluent compared to the lean existing studies.

The total organic carbon (TOC) measures the quantity of total carbon in organic compounds, this time in cassava mill effluent samples. The total organic carbon had a reduction of 92.99% using the powdered *Moringa oleifera* seeds, while the consortium of *Bacillus* and *Pseudomonas* species gave a reduction of 69.17%. However, Okoya *et al*. (2020) reported a similar percentage reduction of 88.9% as in this current study when wastewater was treated using activated *Moringa oleifera* seed powder. The total organic carbon in the CME in this study was indicative of the level of environmental pollution potential, which makes it obvious to treat it before eventual discharge into the environment. However, based on the categorisation by Tchobanoglous and Burton (2003), the TOC values were within the medium class but reduced to acceptable limits.

This study reported a reduction of 99.30% using powdered *Moringa oleifera* seeds and 72.41% using a consortium of *Bacillus* and *Pseudomonas* species in the biological oxygen demand of the cassava mill effluent. Also, the chemical oxygen demand was reduced by 94.25% using powdered *Moringa oleifera* seeds, while the consortium of *Bacillus* and *Pseudomonas* species gave a reduction of 83.34%. The study observed that the ability to remove biological oxygen demand was lower than that of Suhartini et al*.* (2013). The biological oxygen demand and chemical oxygen demand reported in this study were significantly reduced, and they were within the acceptable disposal limits of less than 20 mg/L and 120 mg/L for BOD and COD, respectively (Ibiene et al*.,* 2021; FMoE, 1995). The temperature and pH monitored did not really show marked difference within the studied period. The temperatures reported were within the psychophilic range, which supported the growth of the bacteria isolates used for the study. However, the pH was within the acidic range, which was expected considering the breakdown process (fermentation) involved in the bio-treatment process of CME.

The efficacy of biological treatment of polluted water is determined by its biodegradability index (BI). The biodegradability index value reported in this study ranged from 0.4473 to 0.7506 which corroborates with the earlier reports of Rim-Rukeh (2016) and Ibiene et al. 2021. Rim-Rukeh, (2016) reported a biodegradability index of 0.507 – 0.548 in wastewater from 6 different cassava mills in Nigeria. Ibiene et al. (2021) reported a BI range of between 0.894 and 0.999 in bio-treatment of cassava processing effluent from Umudike using powdered seeds of *Moringa oleifera*.

Also, the report of this study falls within the range of 0.3 to 1.0 which is considered biodegradable. This points to the fact that the cassava mill effluent contained a high quantity of organic matter making biodegradation possible. This study shows that there is a strong positive correlation between BOD and COD which agrees with earlier study (Abdalla and Hammam, 2014).

Conclusion and Recommendation

The results obtained from this study indicated that the set-ups with the powdered *Moringa oleifera* seeds and consortium of *Bacillus* and *Pseudomonas* species effectively improved the turbidity, total organic carbon, biological oxygen demand, and chemical oxygen demand of the cassava mill effluent samples from Ogwa and Ebelle communities of Esan land. This study recommends that cassava mill effluent be treated with powdered *Moringa oleifera* seeds and a consortium of *Bacillus* and *Pseudomonas* species to reduce the level of contamination in the environment and sustain public health of the citizens.

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