# Isolation of Bacteria with Purifying Potential and Application in the Treatment of Effluents from an Artisanal Palm Oil Mill in the Littoral Region of Cameroon

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**How to cite this paper:** Félicité, D.N., Daïna, N.T., Roméo, F.T., Joël, T.S., Fatima, N., Joël, N.N., Yanick, K., Josiane, B., Felix, F., Mpondo, E.M., Beng, V.P. and Marie, T.F. (2021) Isolation of Bacteria with Purifying Potential and Application in the Treatment of Effluents from an Artisanal Palm Oil Mill in the Littoral Region of Cameroon. *Journal of Environmental Protection*, **12**, 462-471.
https://doi.org/10.4236/jep.2021.127029

**Received:** April 11, 2021
**Accepted:** July 6, 2021
**Published:** July 9, 2021



## Abstract

It is with the aim of solving the problem of generating large quantities of effluents from palm oil production in the littoral region of Cameroon that this study was carried out with the general objective of reducing the pollutant load of these effluents by using bacteria. To this end, raw palm oil mill wastewater samples were taken for their characterization by evaluating the *in-situ* (Temperature, pH and (CND) Conductivity) and *ex-situ* (SS (suspended solid), COD (chemical oxygen demand), BOD (biological oxygen demand) and O/F (oil and fat)) parameters. In addition, bacterial isolation and screening were carried out from samples of contaminated soil based on the production of lipolytic enzymes, the degradation of oils and fats and the reduction of the pollutant load. Results revealed that 28 isolates were able to reduce the pollution parameters of palm oil mill effluents of which D17, D22 and D23 seemed to be the best purifying isolates. The characterization of the POME (palm oil mill effluent), basing the temperature, pH, CND, O/F, SS, BOD and COD showed us values greater than the recommended rate. Partial characterization of these isolates revealed that D17 and D23 are bacteria that could reduce the polluting parameters of the effluents belonged to the *Bacillus* sp. genus and D22 to the *Acinetobacter* sp. genus. These results are satisfactory and the bacteria strains obtained could be used in bioremediation.

**Keywords**

Palm Oil, Mill Effluent, Bacteria, Pollution, Cameroon

## 1. Introduction

Water pollution is defined as the degradation of water by altering its natural, physical, chemical and biological properties. It thus disrupts the living conditions of aquatic flora and fauna [1]. To mitigate these negative impacts, it is necessary to put in place methods of treating water before it is discharged or reused for agricultural purposes. Treating wastewater before it is released into the environment is a major challenge for many countries around the world. This difficulty is more accentuated in developing countries which mainly suffer from a lack of capital [2]. This lack of wastewater sanitation has environmental (eutrophication, spread of bad odors), health (development of water-borne diseases) and economic consequences (loss of income for tourism and the high costs of water-borne disease care) [3]. Among the many industrial products likely to play the role of polluting agents, oils and fats constitute a major pollution problem. Vegetable oil mills are characterized by the use of large amounts of water and therefore produce effluents containing high concentration of fat. This poses a real environmental problem because they can behave like hydrocarbons and reduce the passage of oxygen, thus causing the clogging of pipes and the suffocation of aquatic living beings. The addition of microbial lipases and/or the culture of lipase-producing microorganisms in these effluents make it possible to reduce the lipid load and therefore the pollutant load [4]. Palm oil has an annual global production of 50 million tonnes with increasing annual production in Cameroon of 230,000 tonnes in 2010 [5]. Its production generates large quantities of effluents with a high concentration of oil and fat. The natural phenomenon of self-purification cannot therefore by itself achieve a significant reduction in this pollutant load [6]. It is, therefore, necessary to put in place an effective treatment strategy for the wastewater from these oil mills. The treatment of vegetable waters has been tested using several techniques (forced evaporation [7], coagulation-flocculation [8] [9], electro-coagulation [10] [11], aerobic treatment [12], anaerobic treatment [12] [13] and advanced oxidation [14] [15] [16] [17]). In Cameroon, very little work has been carried out by combining a sand filter and a bacterial bed (bacteria isolated from soils contaminated by the latter).

## 2. Material and Methods

### 2.1. Characterization of Palm Oil Mill Effluents (POME)

Samples of effluents from a crude palm oil mill were carried out between November 2020 and January 2021 in the Littoral region of Cameroon. Samples were taken in sterile 1 L bottles. Before the actual sampling, the bottles were washed

three times with the effluent to be sampled in order to maintain the representativeness of the natural environment. All the analyzes and measures necessary to assess the pollution of these waters are governed by Cameroonian standards. *In-situ* parameters measured were pH, temperature and electrical conductivity. Parameters analyzed in the laboratory were Chemical Oxygen Demand (COD), biochemical oxygen demand (BOD), suspended solids (SS), total nitrogen (TN), total phosphorus (TP) and oils and fats.

Temperature, pH and conductivity were measured at the site using a multi-parameter brand Combo HI 98130 from HANNA, instruments equipped with a probe. COD was determined by the "reactor digestion" method [18], the suspended solids (SS) were determined by filtering a volume of wastewater through a cellulose filter (mesh size 0.45 µm) [19]. Biochemical oxygen demand (BOD) was determined by "manometric" method using a WTW brand BOD5 incubator. Oils and fats (O/F) were determined by extracting lipids in a separatory funnel. A volume of 40 ml of crude effluent is mixed with 40 ml of n-hexane for 2 minutes. This extraction is carried out twice in a row. The hexane phase is evaporated using a rotary evaporator under vacuum at a temperature of 100˚C. The tared flask is cooled, and the fat was determined by weighing [20]. The Kjeldahl nitrogen content or even total nitrogen consisting of organic and ammoniacal nitrogen was determined by the Kjeldahl method; Le total phosphorus (TP) is the sum of inorganic phosphorus and organic phosphorus. It was determined after mineralization of the sample [21] by "molybdovanadate" method.

## 2.2. Isolation of Bacteria with Purifying Characteristics

Soil samples were collected using a sterile spatula 0 - 15 cm deep from the soil polluted with POME. The fermentation medium consisted of 0.5% (m/v) peptone; 0.02% (m/v) $MgSO_4$; 0.3% NaCl; 0.1% (m/v) $KH_2P_4$; 0.5% (v/v) olive oil; 0.05% (v/v) of tween 80 to emulsify. The whole was dissolved in distilled water and the pH of the medium was adjusted to 8 by adding 0.3% (m/v) of $Na_2CO_3$. The medium was sterilized at 121˚C for 20 minutes. Once the medium has cooled, the stock solution is obtained after incubation at 30˚C for 24 hours with 5 g of soil in 25 ml of fermentation medium.

The isolation medium had the same composition as the liquid medium in the presence of bacteriological agar. After incubation, the fermented solution was subjected to decimal dilutions and 5 microliters of each fraction were seeded on the surface in Petri dishes according to the protocol used by Fobasso *et al*., In 2019 [22] then incubated at 30˚C for 24 hours. Isolates obtained were individually taken and sub-cultured by the streak method on agar medium and incubated at 30˚C for 24 hours. This operation was repeated until the pure isolates were obtained. Subsequently, these were stored at 4˚C for subsequent analyzes. Regular sub-cultures of isolates were performed every two weeks [23].

## 2.3. Screening of the Best Purifying Isolate

Selecting criteria of the best purifying isolate were based on the relative purifying

performance. The efficacy of each isolate was determined by characterization of the effluent (COD and O/F) before and after fermentation. For this purpose, 3 L of effluent were sterilized at 121˚C for 20 minutes then 60 ml were distributed in sterile 100 ml flasks according to the protocol used by Suseela and Muralidhar in 2018 [24]. Eight percent (8%) of each inoculum containing 106 cells/mL with an optical density of 1.2 at 600 nm was inoculated and then incubated at 30˚C for 5 days with stirring at 150 rpm in the presence of blank.

A bacterial isolate is more effective when it can further reduce the pollutant load present in the effluent. The purification yield was assessed analytically by monitoring the reduction rate of COD and O/F [25]. The calculation of the reduction rate expressed as a percentage was therefore based on the following formula:

$$\text{Reduction}(\%) = 100 - \left[\frac{\text{Craw POME} - \text{Cf}}{\text{Craw POME}} \times 100\right]$$

### 2.4. Partial Characterization of the Best Purifying Isolates

It consisted in partially characterizing the best purifying isolates of our effluents through several tests, namely: phenotypic tests (macroscopic and microscopic identification) and biochemical tests (catalase test).

## 3. Results and Discussion

### 3.1. Characterization of Palm Oil Mill Effluents (POME)

Analysis of the palm oil mills effluent were carried out during the months of November, December (2020) and January 2021 because these are periods of dry seasons in Cameroon when the production of palm oil is very high. During this period, 3 samples at a rate of one sample per month were carried out for the crude effluents.

The palm mill effluents collected were brown in color, oily and bad smelling. The samples had high COD concentrations of 54,960 mg/L, SS of 65,015 ± 2333.8, BOD of 2373.3 ± 262 mg/L, O/F of 605 ± 32.5 mg/L, an NDT of 4.6 ± 0.4 µS/cm, a pH of 4.9 ± 0.4, and a temperature of 51.8˚C ± 1.74˚C. These values are presented in **Table 1** and show that these effluents are highly polluting for the environment. The chemical oxygen demand (COD) and biological oxygen demand ($BOD_5$) wich are the most used polluting parameters were 54,960 ± 11,308.1 mg/L and 2373.33 ± 2623.077 mg/L respectively. COD and $BOD_5$ values obtained during this study are similar to those obtained by Najafpour *et al.* (2006) [26], Alhaji *et al.* (2016) [27] and Nur *et al.* (2017) [28]. However, Jeremiah *et al.* (2014) [25] and Suseela and Muralidhar (2018) [24] obtained higher values. Also, the concentrations of O/F (605 ± 32.5 mg/L) obtained are similar to those obtained by Najafpour *et al.* (2006) [26], and Abdulkarim *et al.* (2011) [29]. These values are greater than those obtained by Suseela and Muralidhar (2018) [24] (209 mg/L) and Jeremiah *et al.* (2014) [25] (190.6 mg/L). Moreover, they are greater than the limit values. These differences are thought to be due to the different species of palm nuts, the frequency and the extraction method used (industrial or artisanal).

**Table 1.** Characterization of the palm oil mill effluents.

| Parameters Values | Units | Mean ± Standard deviation | Minimum Values | Maximum Values |
|---|---|---|---|---|
| **T** | (˚C) | 51.8 ± 1.74 | 45 | 60 |
| **pH** | / | 4.9 ± 0.4 | 4.4 | 5.3 |
| **CND** | µS/cm | 4.6 ± 0.4 | 4.1 | 5.2 |
| **SS** | mg/L | 65,015 ± 2333.8 | 30,866.6 | 82,775.0 |
| **O/F** | mg/L | 605 ± 32.5 | 235 | 800 |
| **COD** | mg/L | 54,960 ± 1308.1 | 46,080.0 | 71,040.0 |
| **BOD** | mg/L | 2373.3 ± 262 | 760 | 5400 |
| **TN** | mg/L | 2963.3 ± 248.7 | 1320 | 5790 |
| **TP** | mg/L | 22,256.6 ± 1357.4 | 9500 | 31,270 |

**T**: temperature; **CND**: conductivity; **SS**: suspended solids; **O/F**: oil and fat; **COD**: chemical oxygen demand; **BOD**: biological oxygen demand; **TN**: total nitrogen; **TP**: total phosphorus.

Also, the quantity of water used during the palm oil manufacturing process, the chemical composition of nuts and crude palm oil could also justify these high values as well as the presence of unrecovered palm oil.

### 3.2. Screening and Isolation of the Best Purifying Isolate

28 bacteria isolates revealed the POME purifying character. 5 were selected for further work because of their ability to produce lipolytic enzymes Figure 1.

### 3.3. Reduction of Oils and Fats

Results obtained showed that these bacterial isolates considerably reduced O/F within 5 days. In sterile POME samples, the rate of reduction of oils and fats are shown in Figure 2.

Figure 2 shows the O/F reduction rates of the isolates during 5 days of incubation in sterile effluent. It emerges from this figure that all these isolates have the capacity to degrade the oils and fats contained in this effluent with the reduction rates of 58.57% (D11), 61.79% (D23), 64.88% (D27), 65.97 % (D22) and 70.81% (D17). However, isolate D17 is the best purifier of biodegradable organic matter because in its presence we obtain a reduction rate of 70.81%. Similar results were obtained by Jeremiah *et al.* (2014) during their studies on the biodegradation of POME by bacteria strains revealed that *Micrococcus luteus* 101PB could reduce oils and fats at the rate of 64.76%, *Stenotrophomonas maltophilia* 102PB at the rate of 67.65%, *Bacillus subtilis* 106PB at the rate of 75.7% and Bacillus cereus 103PB at the rate of 85.14%. The reduction of O/F could be due to the fact that these bacteria produces lipolytic enzymes capable of reducing fat into fatty acids that could be absorb by them.

**Figure 1.** Bacterial isolates from the different samples.

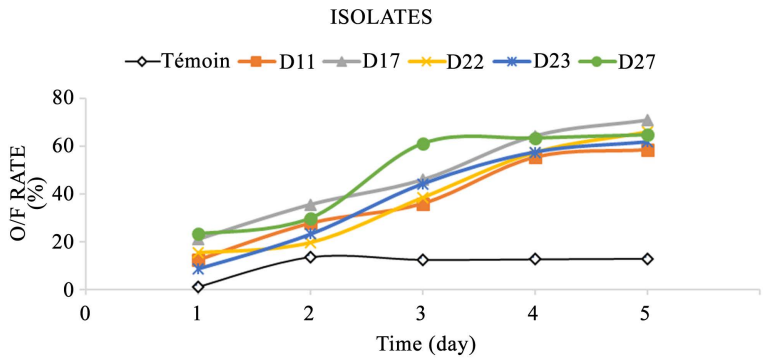


**Figure 2.** Reduction of Oils and Fats (O/F).

Logically, the biological treatment of wastewater contaminated with fats significantly reduces the organic load as well as oils and fats [24] [25] [30] [31] [32].

The difference observed in the abatement rates of the different isolates in these studies was due to the different characteristics of the wastewater because the effluents from oil mills each have their own characteristics [33]. Generally, the microbial degradation of oils and fats is the result of the hydrolysis of oils due to a secretion of lipase and/or esterase, which degrade triglycerides into organic acids (fatty acids) and volatile fatty acids then reduce these molecules via beta oxidation (fatty acid degradation pathway).

### 3.4. Reduction of COD in the Sterile Effluent

Figure 3 shows the COD reduction rates of the isolates during 5 days of incubation in sterile effluent. It emerges from this figure that all these isolates have the capacity to degrade the oxidizable organic matter contained in this effluent. The purifying activities of these isolates after 5 days show the reduction rates of 63.92% (D17), 68.03% (D11), 76.22% (D27), 80.82% (D23), 88.52% (D22) and 2.59% for the test sample. This shows that these isolates are effective in reducing COD in sterile POME. They can then be used in biological processes for treating oxidizable organic materials contained in this effluent.

Similar results regarding COD reduction were obtained by Suseela and Muralidhar (2018). In fact, during their work, they obtained COD abatement rates of 80.28%, 71.08%, 64.83%, 61.86% and 59.26% respectively from *Emericella nidulans*, *Aspergillus niger*, *Trichoderma harzianum*, *Aspergillus fumigatus* and *Trichoderma reesei*. Roux *et al.* in 2005 reported during their work COD abatement

rates of 91.3% by *Rhizopus stolonifer*, 85.3% by *Penicillium* Spp., 84.0% by *Mucor circinelloides f. circinelloides* and 83.8% by *Aspergillus niger*.

The reduction of these pollution parameters is thought to be due to the enzymatic activity exhibited by these isolates. Indeed, they would use these organic materials as a source of energy and carbon necessary for their growth, development and cell synthesis [34]. Also, the abatement rates obtained by these isolates could be explained by the fact that they were isolated from land contaminated by POME and would have adapted to this environment. The biodegradation of oils in the environment is a complex process whose quantitative and qualitative aspects depend on the nature and quantity of oil present, environmental conditions and the constitution of the microbial flora present [25].

### 3.5. Partial Characterization of Selected Isolates

Partial characterization of the best isolates showed that they are all Gram− and Catalase+. Aigbodion *et al.* (2014) [35] have shown through their studies on the microbial populations of POME and their efficiency in the production of biogas that the *Bacillus* genera are Gram−, rod-shaped, white in color and produce lipolytic enzymes. So D17 and D23 could belong to the genus *Bacillus* sp. and D22 to the genus *Acinetobacter* sp. (Table 2).

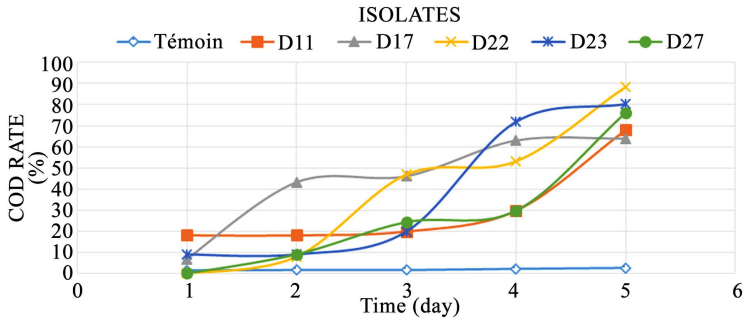


**Figure 3.** Reduction of Chemical Oxygen Demand (COD).

**Table 2.** Partial identification of isolates *D*17, *D*22 and *D*13.

| | ***Characteristics*** | ***D*17** | ***D*22** | ***D*23** |
|---|---|---|---|---|
| ***Macroscopic observations*** | Colonies | Irregular | Regular | Irregulars |
| | Edge | regular | regular | Irregulars |
| | Elevation | Bulging | Bulging | Semi-convex |
| | Area | Brilliant | Brilliant | Rough |
| | Color | Whitish | Pink | Whitish |
| | Breathing mode | Aerobic | Aerobic | Aerobic |
| ***Microscopic observations*** | Form | Stick | Shell | Stick |
| | Mobility | Negative | Positive | Negative |
| | Gram stain | Negative | Negative | Negative |
| ***Biochemical tests*** | Catalase | Positive | Positive | Positive |

## 4. Conclusion

This study revealed that POME is of great capacity of polluting the environment basing on parameters like temperature, COD, BOD, O/F, SS and CND. In addition to that, bacteria strains are likely to be used in the bioremediation process because they have a high rate of reducing the polluting parameters. The rates of reducing O/F by the retained bacteria strains are ranged from 58.57% to 70.81% showing that they could be used in bioremediation to reduce the polluting parameters of POME in Cameroon.

## Acknowledgements

The authors wish to thank his laboratory colleagues for support, researchers of CEREPAH for producing the samples, Dr. NGANGO EBONGUE Georges (head of IRAD branch of Littoral region) for the authorization of the study, and Dr. NSIMI Armand (head of palm oil specialized research) for his technical advice and the concern he had for this study.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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