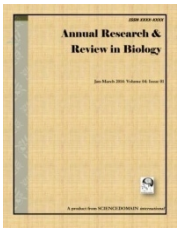

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Fatty Acids and Mineral Profiles of Biodegraded Oil-rich Wastewater

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Authors' contributions

This work was carried out in collaboration between all authors. Author BIA designed the study and wrote the protocol, author ATO performed the statistical analysis, wrote the first draft of the manuscript and managed the literature searches. Author EIA managed the analyses of the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Biodegradation of oil-rich wastewater evidenced with the production of fatty acids was enumerated and qualified.
Study Design: The study site is Falegan restaurant situated within the metropolis of Ado-Ekiti, the capital of Ekiti State. The restaurant is located at about 20km from Ekiti State University, Ado Ekiti.
Place and Duration of Study: Sample: Department of Microbiology, and Department of Chemistry, Ekiti State University, Ado-Ekiti, between September 2010 and August 2011.
Methodology: The oil-rich wastewater and palm oil were cultured using standard methods and the growth measured using gravimetric procedure. Fatty acids enumeration was done using higher performance liquid chromatography. Mineral analyses were carried out on the wastewater samples, using an Atomic Absorption Spectrophotometer, while Potassium and Sodium were analyzed using a Flame Photometer.
Results: In wastewater the percentage weight difference between the first and twelfth days indicated some substantial growth differences in *Pseudomonas* sp. (w) and *Staphylococcus* sp (x) with each having a 100% weight increase. While in palm oil, there were some appreciable increases in weight difference noticed for isolates *Pseudomonas* sp. (j), *Staphylococcus* sp. (r) and *Bacillus* sp. (p), and *Klebsiella* sp. (m) with values of 28.3%, 7.84%, 4.44% and 6.98% respectively. The weight increase of each of the microbial

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cells in palm oil culture was usually lesser than what was obtained in the oil-rich wastewater culture. The largest variation among the microbiological activities in the biodegradation of palm oil fatty acids was obtained on the 7th day. The value on this day was 52.1% which almost doubled the values obtained on day 4 and 24h but almost quadruplet on the 12th day. Generally, *Klebsiella* spp (m) appeared to be the most outstanding in its biochemical use of the minerals; whereas *Staphylococcus* sp. (r) appeared to utilize the minerals minimally.
Conclusion: The biodegradation of the oil-rich wastewater was discussed extensively.

Keywords: Oil-rich wastewater; fatty acids; microbial; biodegradation.

1. INTRODUCTION

Lipid is one of the major organic fractions of restaurant wastes/wastewaters. Other lipid-rich wastes/wastewaters are widely found in certain food processing industries such as the dairy, edible oil and slaughterhouses [1]. Fatty acid is produced when fats are broken down; a carboxylic acid often with a long un-branched aliphatic tail (chain), which is either saturated or unsaturated [2]. Fatty acids are produced by the hydrolysis of the ester linkages in a fat or biological oil (both of which are triglycerides), with the removal of glycerol [3].

Cooking oil is glycerol esters of fatty acids and may be derived from animals or plants [4]. The common types of cooking oil are peanut oil, corn oil and lard. They are used as a heat-transfer medium in frying to generate nicely cooked foods. In frying, cooking oil is heated to a temperature of 170-220ºC. Upon heating, cooking oil may undergo chemical reactions like hydrolysis, oxidation and polymerization. Degradation products such as free fatty acids, hydro peroxides and polymerized triglycerides may be formed [5]. Besides, the viscosity of the cooking oil will increase; its colour will go darker and rancidity will also develop, giving rise to unpleasant flavour as a result of oxidation.

The amount of degradation products (methyl esters) increases with the duration of heating of the cooking oil at high temperatures. Some of these may be used to indicate the degree of degradation of cooking oil. The toxicity of these degradation products (methyl esters) is of health concern [5]. However, there is no definite evidence showing that the use of cooking oil with these degradation products will lead to cancers in humans. It has also been reported that small amounts of acrolein, a toxic substance to experimental animals, may be found in repeatedly heated cooking oil [6]. Its toxic effect on humans is yet to be determined. In this study, the degradation of oil content of wastewater would be extensively discussed.

2. MATERIALS AND METHODS

2.1 Source of Bacteria

The bacteria isolates used in this study have been previously described [7]. Isolates were recovered from oil-rich wastewater samples from a restaurant in Ado-Ekiti, Nigeria. The isolates had previously been identified on the basis of standard physiological, biochemical, and serological assays. The isolates include one hundred and eleven bacteria belonging to the following genera; *Pseudomonas*, *Staphylococcus*, *Enterococcus, Escherichia*, *Bacillus*, *Streptococcus* and *Klebsiella*. These organisms were obtained from wastewater after dish

washing (with soap solution; 5g/100ml detergent in water), first and second rinsing of the dishes, and run-off into open sewers along the drainage.

2.2 Growth of Bacteria Isolates in Wastewater and Palm Oil

A pure colony of each isolate was inoculated on sterile Tributyrin agar (TBA) and incubated at 37ºC for 24h. Lipolytic activity of each isolate was confirmed by the development of zone (s) of clearance around each colony [8]. Subsequently, 0.1 ml of known concentration of organism was inoculated into 100ml sterile oil-rich wastewater and 10 ml of known concentration of inoculum into 90 ml of palm oil respectively. The mixture was incubated at ambient temperature and growth monitored for 12days. All the experiments were carried out in triplicates and conducted twice.

2.3 Estimation of Fatty Acids in Wastewater Samples

A known quantity of wastewater sample was warmed in sterile round bottom flask in a water bath (Model CS200) at 43ºC and shaken thoroughly to ensure the fats and oils present become uniformly distributed and homogenous throughout sampling.

2.4 Extraction and Methylation of Oil from the Wastewater Samples

Fat was extracted from the wastewater samples by adding 60ml concentrated HCl to 60ml of the wastewater. This was followed by the addition of 60ml diethyl ether and 60ml of n-hexane [9]. The resulting mixture was thoroughly shaken and transferred to a separating funnel. It was allowed to stand for 30min. The solvent layer was separated and removed from the fat at a low temperature (7ºC). The extracted oil from the wastewater samples was transferred into a test tube and 4ml of 0.5M methanolic sodium hydroxide (20g of NaOH in 1liter methanol) was added and heated on a steam bath for 5mins until it globulized into solution. Five millilitre of BF_3/MeOH (14% Boron trifluoride; 86% Methanol, 100 ml) was added onto the mixture and boiled for 2min [10]. This mixture was then transferred into a 250ml separating funnel and 30ml of petroleum ether (40-60ºC boiling range) was added. Twenty millilitre of saturated sodium chloride solution were later added and shaken vigorously. The lower layer of aqueous methanol was allowed to separate, drained off and discarded. The petroleum ether layer was then filtered through a Whatman No.1 filter paper into a 50ml beaker. The solvent was evaporated on a steam bath to a final volume of 10ml. The methyl esters were used for HPLC analysis [10].

2.5 Characterization and Enumeration of Fatty Acids in the Methylated Oils

The AKTA basic 10/100 high performance liquid chromatography (Amersham Pharmacia Biotech, Sweden) was used for the fatty acids determination. The analysis was carried out using Supercosil LC-18 column, 25cm x 4.6mm ID, 5µm particles. The mobile phase consisted of acetonitrile: acetone (59:41; v/v) with a flow rate of 1.0ml/min and fatty acids were detected using a UV (UV-900) detector. Ten microlitre sample of the fresh and biodegraded palm oil was injected for each run. The fatty acid components in the test samples were identified by comparing retention times to those of the standards. Each fatty acid in the test sample was expressed in percent of the total fatty acids present. The methyl esters of lauric, myristic, palmitic, stearic, oleic, linoleic, linolenic and arachidic acids were used as standards for identification [9].

Calculation: % Fatty acid = $\frac{\text{Peak area of the sample x conc. of fatty acid x diluting factor}}{\text{Peak area of the standard}}$

2.6 Mineral Analyses

The mineral analyses were carried out on the wastewater samples, using an Atomic Absorption Spectrophotometer (α-4-Baird) with specific lamp for each metal (Zn, Fe, Cu, Co, Pb, Mn, Mg and Ca), while Potassium and Sodium were analyzed using a Flame Photometer (BUCK 2010 VGP AAS) [10]. The standard as well as the sample for each metal was aspirated into the flame. The respective concentrations in mg/l were read for each sample while the absorbance of the standards was recorded.

3. RESULTS

3.1 Growth of Lipolytic Organisms in Wastewater

Half the number of microbes that grew on Tributyrin agar (16/32, 50%) recorded maximum growth after five (5) days of biodegradation of the wastewater. *Staphylococcus* sp. (p) and *Pseudomonas* sp. (j) grew very well (weight > 0.10 mg) after 6 days. On day 12, 40.6% of the isolates still had twice the initial cell mass after 24h incubation, with *Pseudomonas* sp. (dd) recording over 66.7% weight increase (Table 1). Only three isolates, *Klebsiella* sp. (m), *Pseudomonas* spp. (j) and (w) showed appreciable growth rates (≥ 0.02 mg/day) within 6 days. The growth pattern of the lipolytic microbes in the wastewater varied. The percentage weight difference between the first and twelfth days indicated some substantial growth differences in *Pseudomonas* sp. (w) and *Staphylococcus* sp (x) with each having one hundred percent (100%) weight increase. There was also an appreciable increase in the weight of *Pseudomonas* spp. (dd), with 66.7% weight increase (Table 1). Hence, the most effective lipolytic bacteria isolates in the oil-rich wastewater were *Pseudomonas* sp. (dd), *Pseudomonas* sp. (w) and *Staphylococcus* sp. (x) (Fig.1).

3.2 Growth of Lipolytic Organisms in Palm Oil

The weights of the isolates after 24h incubation ranged between 0.33mg and 0.60mg and the final weights on the 12^{th} day were between 0.25mg and 0.51mg. Generally, the optimum growth in all the thirty two (32) lipolytic microbial isolates in the fresh oil was between the fifth and seventh days (Table 2). The growth rate per day ranged between 0.02mg/day in *Pseudomonas* sp. (n), to 0.03mg/day in *Klebsiella* sp. (m), 0.04mg/day in *Pseudomonas* sp. (j), 0.04mg/day in *Staphylococcus* sp. (r), and 0.05mg/day in *Bacillus* sp. (p). Comparing the weights on the first day and the twelfth day, there were some appreciable increases in the weight difference noticed for *Pseudomonas* sp. (j), *Staphylococcus* spp. (r), *Bacillus* (p), and *Klebsiella* sp. (m) with values of 28.3%, 7.84%, 4.44% and 6.98% respectively. The weight increase of each of the microbial cells in palm oil culture was usually lesser than what was obtained in the oil-rich wastewater culture.

In selecting bacteria isolates for further studies, the weight of each microbe on the twelfth day was compared to the weight after the first day of incubation. The percentage weight difference was between -64% and 28.3%, showing significant difference at $p \leq 0.05$. The bacteria isolates selected, *Pseudomonas* spp. (j) and (n), *Klebsiella* sp. (m), *Bacillus* sp. (p) and *Staphylococcus* sp. (r), grew well in fresh palm oil with the percentage weight difference of 28.3%, 2.04%, 6.98%, 4.44% and 7.84% respectively (Fig. 2). The decomposition of

dietary oil was primarily dependent on the lipolytic ability of the various bacteria as was observed by the different cell weights.

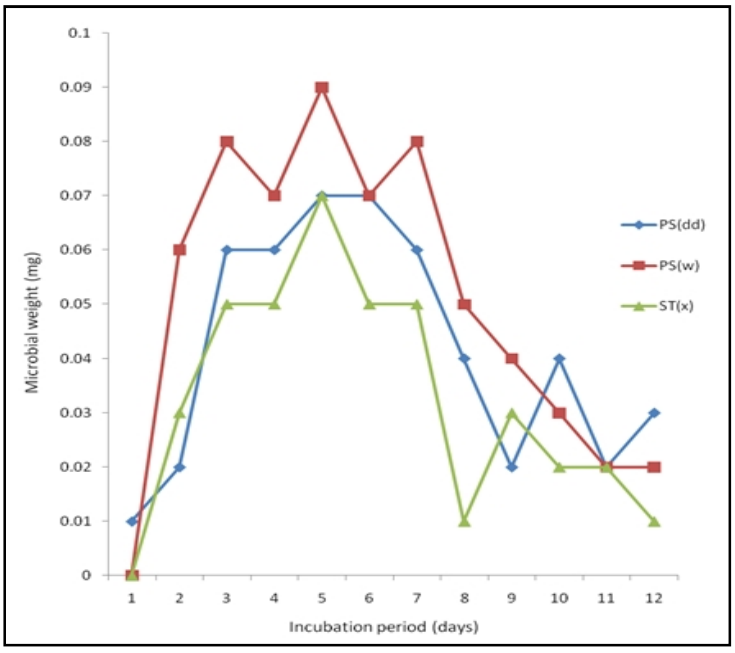

Fig 1. Growth of selected most effective lipolytic bacteria isolates in oil-rich wastewater

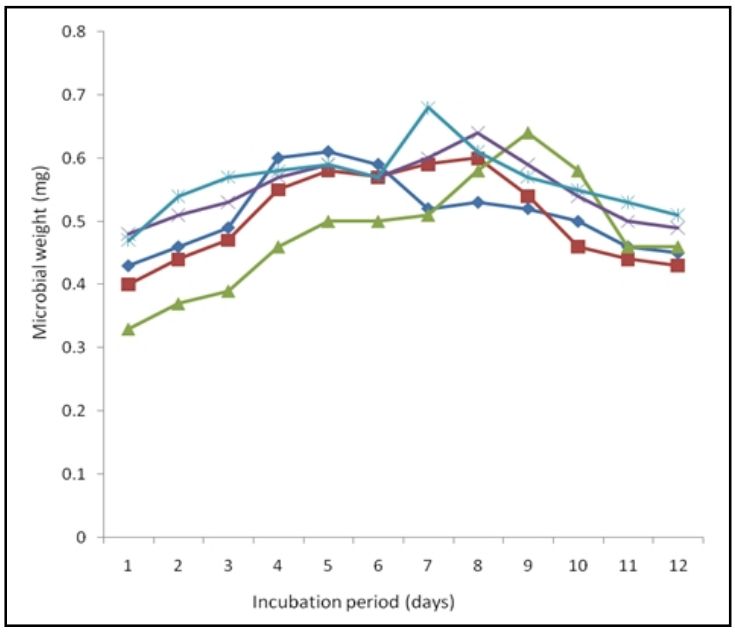

Fig. 2. Growth of selected most effective lipolytic bacteria isolates in fresh palm oil

Table 1. Growth (mg) of lipolytic isolates[1] in wastewater

Code	Isolates	Days of incubation												% weight*
		1	2	3	4	5	6	7	8	9	10	11	12	difference
$D2_2$	*Bacillus* sp. (p)	0.04	0.02	0.03	0.05	0.07	0.09	0.07	0.04	0.03	0.04	0.04	0.03	-33.3%
A_2	*E. coli* (v)	0.01	0.03	0.06	0.08	0.08	0.06	0.06	0.04	0.03	0.03	0.01	0.01	0%
$D1_7$	*E. coli* (f)	0.03	0.03	0.06	0.06	0.07	0.05	0.05	0.03	0.04	0.03	0.03	0.03	0%
$D2_{10}$	*E. coli* (t)	0.02	0.02	0.06	0.09	0.09	0.08	0.08	0.04	0.02	0.02	0.02	0.02	0%
$D2_{11}$	*Enterobacter* sp. (a)	0.02	0.02	0.06	0.06	0.05	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0%
$D3_5$	*Enterobacter* sp. (b)	0.01	0.03	0.06	0.07	0.07	0.08	0.08	0.04	0.02	0.02	0.02	0.02	+50.0%
$S1_{10}$	*Enterobacter* sp. (o)	0.01	0.05	0.06	0.06	0.08	0.09	0.09	0.06	0.04	0.03	0.02	0.02	+50.0%
$D2_{21}$	*Klebsiella* sp. (m)	0.01	0.05	0.05	0.07	0.09	0.09	0.09	0.05	0.04	0.04	0.03	0.02	+50.0%
$D3_3$	*Klebsiella* sp. (d)	0.02	0.02	0.03	0.04	0.06	0.07	0.07	0.03	0.01	0.02	0.01	0.01	-100%
A_6	*Pseudomonas* sp. (s)	0.01	0.01	0.04	0.04	0.05	0.06	0.06	0.03	0.01	0.01	0.01	0.01	0%
A_{13}	*Pseudomonas* sp. (w)	0.00	0.06	0.08	0.07	0.09	0.07	0.08	0.05	0.04	0.03	0.02	0.02	+100%
B_{12}	*Pseudomonas* sp. (aa)	0.02	0.01	0.03	0.03	0.05	0.06	0.06	0.02	0.02	0.03	0.02	0.03	+33.3%
C_{10}	*Pseudomonas* sp. (dd)	0.01	0.02	0.06	0.06	0.07	0.07	0.06	0.04	0.02	0.04	0.02	0.03	+66.7%
$D1_{15}$	*Pseudomonas* sp. (e)	0.03	0.04	0.09	0.08	0.10	0.10	0.08	0.06	0.03	0.03	0.03	0.03	0%
$D1_{18}$	*Pseudomonas* sp. (y)	0.04	0.02	0.05	0.06	0.08	0.08	0.08	0.02	0.02	0.01	0.01	0.02	-100%
$D1_{19}$	*Pseudomonas* sp. (g)	0.03	0.04	0.07	0.06	0.08	0.05	0.05	0.04	0.03	0.02	0.02	0.01	-200%
$D1_{23}$	*Pseudomonas* sp. (ee)	0.01	0.02	0.02	0.05	0.06	0.06	0.05	0.03	0.01	0.02	0.01	0.01	0%
$D2_4$	*Pseudomonas* sp. (j)	0.01	0.02	0.02	0.08	0.09	0.11	0.10	0.06	0.06	0.03	0.02	0.02	+50%
$D3_7$	*Pseudomonas* sp. (k)	0.02	0.02	0.03	0.04	0.06	0.08	0.08	0.03	0.03	0.03	0.02	0.01	-100%
$S2_7$	*Pseudomonas* sp. (n)	0.01	0.07	0.07	0.06	0.07	0.10	0.07	0.04	0.03	0.04	0.02	0.02	+50.0%
$S2_2$	*Serratia* sp .(h)	0.03	0.03	0.04	0.05	0.06	0.08	0.06	0.03	0.03	0.04	0.03	0.03	0%
$U2_3$	*Serratia* sp. (q)	0.01	0.04	0.05	0.05	0.05	0.09	0.07	0.04	0.03	0.02	0.02	0.01	0%
A_7	*Staphylococcus* sp. (z)	0.03	0.01	0.07	0.07	0.08	0.06	0.06	0.03	0.02	0.01	0.01	0.01	-200%
A_{17}	*Staphylococcus* sp. (l)	0.01	0.03	0.08	0.08	0.09	0.09	0.08	0.04	0.02	0.01	0.01	0.01	0%
B_8	*Staphylococcus* sp. (u)	0.02	0.04	0.05	0.08	0.08	0.07	0.07	0.02	0.03	0.02	0.02	0.02	0%
B_{15}	*Staphylococcus* sp. (c)	0.01	0.03	0.03	0.05	0.07	0.07	0.08	0.05	0.03	0.03	0.02	0.02	+50.0%
$D1_8$	*Staphylococcus* sp. (i)	0.01	0.01	0.03	0.03	0.05	0.06	0.06	0.03	0.03	0.04	0.03	0.02	+50.0%
$D1_{20}$	*Staphylococcus* sp. (r)	0.01	0.03	0.05	0.05	0.05	0.04	0.03	0.02	0.02	0.01	0.01	0.02	+50.0%
$D2_{13}$	*Staphylococcus* sp. (bb)	0.01	0.02	0.03	0.04	0.05	0.05	0.04	0.03	0.03	0.02	0.02	0.02	+50.0%
$D3_1$	*Staphylococcus* sp. (x)	0.00	0.03	0.05	0.05	0.07	0.05	0.05	0.01	0.03	0.02	0.02	0.01	+100%
$S1_9$	*Staphylococcus* sp. (cc)	0.01	0.02	0.03	0.04	0.06	0.06	0.03	0.01	0.01	0.01	0.02	0.02	+50.0%
$S2_5$	*Staphylococcus* sp. (ff)	0.01	0.02	0.03	0.04	0.06	0.04	0.04	0.03	0.03	0.01	0.01	0.01	0%

[1]Zero point one (0.1) ml of known concentration of organism in 100ml wastewater
**Difference in percentage weight after 24h and 12days*

Table 2. Growth (mg) of bacterial isolates[1] in Palm oil

Code	Isolates	Days of incubation												% weight*
		1	2	3	4	5	6	7	8	9	10	11	12	difference
$D2_2$	*Bacillus* sp. (p)	0.43	0.46	0.49	0.60	0.61	0.59	0.52	0.53	0.52	0.50	0.46	0.45	+4.44%
A_2	*E. coli* (v)	0.41	0.50	0.52	0.53	0.54	0.55	0.54	0.51	0.49	0.44	0.37	0.36	-13.9%
$D1_7$	*E. coli* (f)	0.46	0.52	0.55	0.57	0.62	0.60	0.55	0.56	0.56	0.49	0.43	0.41	-12.2%
$D2_{10}$	*E. coli* (t)	0.48	0.52	0.54	0.57	0.54	0.56	0.59	0.54	0.49	0.44	0.36	0.35	-37.1%
$D2_{11}$	*Enterobacter* sp. (a)	0.41	0.46	0.47	0.60	0.62	0.61	0.46	0.39	0.35	0.33	0.26	0.25	-64.0%
$D3_5$	*Enterobacter* sp. (b)	0.52	0.57	0.59	0.62	0.62	0.62	0.49	0.43	0.42	0.39	0.38	0.37	-40.5%
$S1_{10}$	*Enterobacter* sp. (o)	0.59	0.59	0.62	0.64	0.63	0.62	0.55	0.56	0.52	0.48	0.43	0.41	-43.9%
$D2_{21}$	*Klebsiella* sp. (m)	0.40	0.44	0.47	0.55	0.58	0.57	0.59	0.60	0.54	0.46	0.44	0.43	+6.98%
$D3_3$	*Klebsiella* sp. (d)	0.60	0.61	0.63	0.64	0.66	0.65	0.61	0.55	0.56	0.49	0.44	0.39	-53.8%
A_6	*Pseudomonas* sp. (s)	0.43	0.50	0.54	0.55	0.56	0.56	0.50	0.50	0.50	0.47	0.42	0.41	-4.88%
A_{13}	*Pseudomonas* sp. (w)	0.46	0.54	0.56	0.57	0.57	0.60	0.56	0.56	0.56	0.47	0.43	0.40	-15.0%
B_{12}	*Pseudomonas* sp. (aa)	0.58	0.60	0.62	0.65	0.65	0.65	0.64	0.54	0.49	0.42	0.39	0.38	-52.6%
C_{10}	*Pseudomonas* sp. (dd)	0.51	0.55	0.57	0.59	0.61	0.61	0.66	0.54	0.53	0.46	0.44	0.42	-21.4%
$D1_{15}$	*Pseudomonas* sp. (e)	0.48	0.50	0.52	0.55	0.56	0.55	0.53	0.48	0.45	0.40	0.38	0.37	-29.7%
$D1_{19}$	*Pseudomonas* sp. (g)	0.59	0.58	0.62	0.64	0.64	0.62	0.58	0.61	0.61	0.56	0.49	0.46	-28.3%
$D2_4$	*Pseudomonas* sp. (j)	0.33	0.37	0.39	0.46	0.50	0.50	0.51	0.58	0.64	0.58	0.46	0.46	+28.3%
$D3_7$	*Pseudomonas* sp. (k)	0.45	0.46	0.48	0.54	0.59	0.58	0.53	0.51	0.48	0.44	0.37	0.36	-25.0%
$S2_7$	*Pseudomonas* sp. (n)	0.48	0.51	0.53	0.57	0.59	0.57	0.60	0.64	0.59	0.54	0.50	0.49	+2.04%
$D1_{18}$	*Pseudomonas* sp. (y)	0.53	0.54	0.58	0.62	0.63	0.62	0.58	0.48	0.46	0.40	0.36	0.34	-55.9%
$D1_{23}$	*Pseudomonas* sp. (ee)	0.48	0.49	0.50	0.51	0.54	0.57	0.60	0.58	0.53	0.48	0.39	0.38	-26.3%
$S2_2$	*Serratia* sp .(h)	0.46	0.48	0.50	0.52	0.56	0.57	0.55	0.55	0.46	0.42	0.39	0.38	-21.1%
$U2_3$	*Serratia* sp. (q)	0.45	0.47	0.49	0.54	0.56	0.54	0.53	0.50	0.48	0.39	0.35	0.33	-36.4%
A_7	*Staphylococcus* sp. (z)	0.55	0.57	0.59	0.62	0.59	0.59	0.55	0.53	0.51	0.47	0.42	0.41	-34.1%
A_{17}	*Staphylococcus* sp. (l)	0.46	0.57	0.58	0.59	0.61	0.60	0.57	0.52	0.53	0.47	0.43	0.41	-12.2%
B_8	*Staphylococcus* sp. (u)	0.42	0.49	0.53	0.54	0.57	0.56	0.53	0.50	0.50	0.44	0.40	0.38	-10.5%
B_{15}	*Staphylococcus* sp. (c)	0.59	0.58	0.61	0.65	0.66	0.65	0.64	0.57	0.57	0.52	0.46	0.45	-31.1%
$D1_8$	*Staphylococcus* sp. (i)	0.51	0.53	0.56	0.59	0.61	0.60	0.57	0.54	0.57	0.49	0.47	0.44	-15.9%
$D1_{20}$	*Staphylococcus* sp. (r)	0.47	0.54	0.57	0.58	0.59	0.57	0.68	0.61	0.57	0.55	0.53	0.51	+7.84%
$D2_{13}$	*Staphylococcus* sp. (bb)	0.50	0.54	0.56	0.57	0.57	0.65	0.57	0.52	0.48	0.40	0.35	0.33	-51.5%
$D3_1$	*Staphylococcus* sp. (x)	0.52	0.58	0.61	0.63	0.63	0.59	0.52	0.48	0.46	0.41	0.40	0.39	-33.3%
$S1_9$	*Staphylococcus* sp. (cc)	0.52	0.55	0.58	0.62	0.64	0.60	0.65	0.58	0.54	0.50	0.47	0.46	-13.0%
$S2_5$	*Staphylococcus* sp. (ff)	0.60	0.60	0.62	0.65	0.65	0.64	0.63	0.58	0.56	0.51	0.45	0.42	-42.9%

[1]Ten milliliter of known concentration of inoculum in 90 ml of palm oil
**Difference in percentage weight after 24h and 12days*

3.3 Microbial Degradation of Lipids

3.3.1 Production of fatty acids

The utilization of the dietary oil as substrate may be explained by fatty acids profiling of the extracted oil from the wastewater culture over specific incubation periods (24h, 4th, 7th, 9th and 12th days) where outstanding microbial growth was experienced (Tables 3a-3f). It was observed that the amount of fatty acids used up by the microbial cells was similar with the quantity released into the environment (Table 3e).

Twenty three out of 32 fatty acids had negative values when degraded by *Klebsiella* sp. (m). Two of these acids were not detected (ND); three had zero values at both initial and final readings. Three FAs had positive balance while one had both the initial and utilized (final) values equal, given a balanced value. The position results came from each of the three major types of fatty acids (FAs): the saturated fatty acid (SFA) of C18:0 had a positive result 0.7326 (13.1%), the mono-unsaturated fatty acid (MUFA) of C18:1 (Cis-6) gave a positive change of 0.8393 (15.0%) and di-unsaturated fatty acid (DUFA) of C20:2 (Cis-11, 14) with a value of 0.0004 (2.74%). In the case of the negative responses these varied values cut across the three major types and other forms of FAs in *Klebsiella* sp. (m), with the total negative and positive values at -1.5669 and +1.5719 respectively; taking cognizance of these two arithmetical signs, the grand total value equal +0.0050. When this one is placed all over 3.1388, the percentage value equals 0.16%. This percentage value of 0.16% is highly negligible and this is invariably the value of FAs that cannot be accounted for under the *Klebsiella* sp. (m) degradation activities in the palm oil at 24h. Since 0.16% is so low that it can be said to be negligible, therefore, it could be said that, the negative and the positive values are visually of equivalent amount.

With *Pseudomonas* spp (j), one fatty acid was not detected, four FAs had zero at both the initial and final values. Six FAs had negative (-ve) balance while twenty one were positive out of 32 FAs. Negative results were recorded for SFA, MUFA and DUFA as follows: SFAs of C14:0, C16:0 and C18:0 had negative values of – 0.0372 (4.46%), -0.5609 (1.44%) and – 1.1216 (20.1%) respectively showing a progressive increase from 14:0 to 18:0; MUFAs of C14:1 (Cis-9) and C16:1(Cis-9) had negative values of –0.0045 (13.3%) and -0.0119 (9.86%) respectively while the only DUFA of C18:2 (Cis-9,12) had a value of -0.2331 (1.78%).

In the case of positive responses, the results also cut across the three major and other forms of FAs in *Pseudomonas* sp. (j). The critical look on the microbiological degradation under *Pseudomonas* spp. (j) showed that the total positive and negative values were + 1.9689, and -1.9692 respectively; taking cognizance of these two arithmetical signs, the grand total value equalled -0.0003. When this is placed all over 3.9381, the percentage value is 0.0089%. This implied that the positive and negative values of the FAs under *Pseudomonas* sp. (j) at 24h are almost of equivalent amounts and there was total virtual utilization of FAs under *Pseudomonas* sp. (j) microbiological degradation of the palm oil; the difference was just 0.0003% or overall percentage difference of 0.0089%. Careful observation of other values under *Pseudomonas* sp. (n), *Bacillus* sp. (p) and *Staphylococcus* sp. (r) followed similar trends as observed with *Klebsiella* sp. (m) and *Pseudomonas* sp. (j). Under *Pseudomonas* sp. (n), the total difference was 0.00033713 approximately 0.00%; *Bacillus* sp. (p), with total difference of 0.00529189, approximately 0.53% and for *Staphylococcus* sp. (r), the total difference was -0.00015568, approximately 0.00% (Table 3a).

On day 4, the percentages of fatty acids of the microbiologically degraded palm oil were shown in Table 4.3b. With *Klebsiella* sp. (m), one FA was ND, four had zero at both initial and final values. Six FAs had negative (-ve) balance while twenty one out of 32 FAs were positive. Negative values were rerecorded for SFA, MUFA and DUFA as follows; SFAs of 14:0, 16:0, C18:0 having values of -0.8298 (0.71%),-1.9231 (45.8%) and -0.1463 (3.49%) respectively showing sudden increase in 16.0; MUFA of 14:1 (Cis-9), 16:1 (Cis-9) and 18:1 (trans-9) with negative values of 0.0298 (0.70%), 0.0518 (1.23%) and 0.0083 (0.20%) respectively. The positive response values cut across the three major and other forms of FAs in *Klebsiella* sp. (m). The critical look on the microbiological degradation under *Klebsiella* sp. (m) showed that, the total positive and negative values at +2.0986 and 2.0992 respectively took cognizance of these two arithmetical signs with the grand total value as +0.0006. When this is placed all over 4.1978, the percentage value equals 0.01%. This implied that, the positive and negative values of the FAs under *Klebsiella* sp. (m) on day 4 are of almost of equivalent amount and there was total utilization of FAs under *Klebsiella* sp. (m) microbiological degradation of palm oil; the difference was just +0.0006% or overall percentage difference of 0.01%.

With *Pseudomonas* sp. (j), one FA was ND, four had zero at both the initial and final values, four FAs had negative (-ve) balance while twenty three were positive. Negative values were recorded for SFA and MUFA as follows: SFAs of 14:0; 16:0 and 18:0 having values of 0.0816 (0.97%), -3.4691 (41.4% and -0.6375 (7.61%) respectively, showing sudden increase of 16:0 as shown in case of 16:0 of *Klebsiella* sp. (m) on day 4; MUFA of 16:1 (Cis-9) showed negative value of -0.0833 (0.99%). The positive response values cut across the three major and other forms of FAs in *Pseudomonas* sp. (j). The critical look on the microbiological degradation under *Pseudomonas* sp. (j) was that, the total positive and negative values was +4.1880 and -4.1882 respectively; taking cognizance of these two arithmetical signs, the grand total value was +0.0002. When this is placed allover 8.3762, the percentage value equaled 0.002%. This implied that the positive and negative values of the FAs under *Pseudomonas* sp. (j) on day 4 were almost of an equivalent amount and there was total virtual utilization of FAs under *Pseudomonas* sp. (j) microbiological degradation of palm oil; the difference was just -0.0002% or overall percentage difference of 0.002%.

Careful observation of the activities of *Bacillus* sp. (p) and *Staphylococcus* sp. (r) followed the trend as discussed under *Klebsiella* sp. (m) and *Pseudomonas* sp. (j). Under *Pseudomonas* sp. (n) the total difference was 0.00004766 approximately 0.00%; *Bacillus* sp. (p) with total difference 0.00240119 approximately 0.24% and *Staphylococcus* sp. (r), total difference 0.00004087 approximately 0.00% (Table 3b).

On day 7, the percentages of fatty acids of the microbiologically degraded palm oil were shown in Table 3c. *Klebsiella* sp. (m) produced five FAs with positive (+ve) balance while twenty two of these acids were negative. The positive values were recorded for SFA, MUFA and DUFA as follows: SFAs of 12: 0 and 14:0 have values of 0.0014 (0.04%) and 0.4182 (13.4%) respectively with increase in value from 12:0 to 14:0; MUFA of 18:1(Cis-6) and 18:1 (Cis-9) have values of 1.6920 (59.1%) and 1.4754 (47.2%) respectively, showing a decrease in value from Cis-6 to Cis-9 of 18:1; DUFA of 18:2 (Cis-9,12) with value of 1.6815 (53.7%). The negative response values cut across the three major and other forms of FAs in *Klebsiella* sp. (m). The critical look on the microbiological degradation under *Klebsiella* sp. (m) was that, total negative and positive values were -1.5569 and +1.5719 respectively; taking cognizance of these two arithmetical signs, the grand total value is -0.005. When this is placed all over -1.5569, the percentage value equaled 0.32%. This implied that the positive and negative values of the FAs under *Klebsiella* sp. (m) on day 7 were almost of

equivalent amount and there was total utilization of the FAs under *Klebsiella* sp. (m) microbiological degradation of palm oil; the difference was just 0.005% or overall percentage difference of 0.00321151 approximately 0.00%. When *Pseudomonas* sp. (j) was considered, one FA was ND; four had zero values at both initial and final readings. Three FAs had negative (-ve) balance while 24 were positive out of 32 FAs parameters. Negative values were recorded for SFA as follows: 14:0, 16:0 and 18:0, have values of -0.8921 (5.55%), -5.0455 (31.4%) and -2.0947 (13.0%) respectively, showing 16:0 with higher value of FAs. The positive response values cut across the three major and other forms of FAs in *Pseudomonas* sp. (j). The critical look on the microbiological degradation under *Pseudomonas* sp. (j) was that, total negative and positive values were -8.0323 and +8.0322 respectively; taking to cognizance of these two arithmetical signs, the grand total value equal + 0.0001. When this is placed all over 16.0645, the percentage value equal 0.001%. This implied that, the positive and negative values of the FAs under *Pseudomonas* sp. (j) on day 7 are almost of equivalent amount and there was total virtual utilization of FAs under *Pseudomonas* sp. (j) microbiological degradation of palm oil; the difference was just 0.0001% or overall percentage difference of 0.000622491 approximately 0.001%.

Careful observation of activities of *Pseudomonas* sp. (n), *Bacillus* sp. (p) and *Staphylococcus* sp. (r) followed the trend as discussed under *Klebsiella* sp. (m) and *Pseudomonas* sp. (j). Under *Pseudomonas* sp. (n), the total difference was 0.00001769 or approximately 0.00%; *Bacillus* sp. (p), total difference was 0.00004918 or approximately 0.00% and *Staphylococcus* sp. (r) total difference was 0.00141376 or approximately 0.14% (Table 3c). The summary of Tables 3a to 3e is shown in Table 3f. Without detailed values in Tables 3a to 3e, Table 3f contains the mean, SD and CV%. This does not include day 9 because only *Pseudomonas* sp. (j) had any result. The purpose of calculating the mean, SD and CV% is to know the comparative variation in the total negative and total positive values for all the days among the various microbiological organisms. Day 12 showed the least variation of CV% of 15.3. The next to it is 24 h incubation period with a CV% of 30.4 which about doubles the value of 15.3 recorded on day 12. The 4^{th} day gave a CV% value of 34.6 which is close to the value for 24h but more than double the value for the 12 days. The largest variation among the microbiological activities in the biodegradation of palm oil fatty acids is obtained on the 7^{th} day. The value on this day was 52.1% which almost doubled the values obtained on day 4 and 24h but almost quadruplet of 12^{th} day.

Table 3a. Fatty acids content (%) of microbiological degradation products of palm oil (24h)

S/N	Fatty Acids	Inoculated oil				
		***Klebsiella* sp. (m)**	***Pseudomonas* sp. (j)**	***Pseudomonas* sp. (n)**	***Bacillus* sp. (p)**	***Staphylococcus* sp. (r)**
1	C5:0	ND – 0.0000 = ND	ND – 0.0000 = ND	ND – 0.0000 = ND	ND – 0.0000 = ND	ND – 0.0000 = ND
2	C6:0	0.0000 – ND = ND	0.0000 – ND = ND	0.0000 – ND = ND	0.0000 – ND = ND	0.0000 – ND = ND
3	C8:0	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
4	C10:0	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
5	C12:0	0.0111 – 0.0114 = - 0.0003	0.0111 – 0.0078 = 0.0033	0.0111 – 0.0117 = 0.0006	0.0111 – 0.0135 = - 0.0024	0.0111 – 0.0112 = - 0.0001
6	C14:0	0.8346 – 0.8862 = - 0.0516	0.8346 – 0.8718 = - 0.0372	0.8346 – 0.9796 = - 0.1450	0.8346 – 0.9152 = - 0.0806	0.8346 – 0.9440 = - 0.1094
7	C14:1(Cis-9)	0.0338 – 0.0346 = - 0.0008	0.0338 – 0.0383 = - 0.0045	0.0338 – 0.0354 = - 0.0016	0.0338 – 0.0411 = - 0.0073	0.0338 – 0.0587 = - 0.0249
8	C16:0	38.9032 – 39.6688 = - 0.7656	38.9032 – 39.4641 = - 0.5609	38.9032 – 39.1231 = - 0.2199	38.9032 – 40.7051 = - 1.8019	38.9032 – 38.6949 = 0.2083
9	C16:1(Cis-9)	0.1207 -0.1238 = - 0.0031	0.1207 -0.1326 = - 0.0119	0.1207 – 0.1267 = - 0.0060	0.1207 – 0.1468 = - 0.0261	0.1207 – 0.1300 = - 0.0093
10	C18:0	5.5831 – 4.8505 = 0.7326	5.5831 – 6.7047 = - 1.1216	5.5831 – 6.3086 = 0.7255	5.5831 – 5.8549 = - 0.2718	5.5831 – 5.8675 = - 0.2844
11	C18:1(Trans-6)	0.0518 – 0.0531 = - 0.0013	0.0518 – 0.0364 = 0.0154	0.0518 – 0.0543 = - 0.0025	0.0518 – 0.0630 = - 0.0112	0.0518 – 0.0558 = - 0.0040
12	C18:1(Cis-6)	5.5851 – 4.7438 = 0.8393	5.5851 – 4.6354 = 0.9497	5.5851 – 5.9149 = - 0.3298	5.5851 – 5.6023 = - 0.0172	5.5851 – 4.9883 = 0.5968
13	C18:1(Trans-9)	0.0034 – 0.0034 = 0.0000	0.0034 – 0.0024 = 0.0010	0.0034 – 0.0035 = - 0.0001	0.0034 – 0.0041 = - 0.0007	0.0034 – 0.0036 = - 0.0002
14	C18:1(Cis-9)	34.6824 – 35.1607 = - 0.4783	34.6824 – 33.9996 = 0.6828	34.6824 – 33.5345 = 1.1479	34.6824 – 33.0349 = 1.6475	34.6824 – 34.3058 = 0.3766
15	C18:1(Trans-11)	0.0590 – 0.0605 = - 0.0015	0.0590 – 0.0415 = 0.0175	0.0590 – 0.0619 = - 0.0029	0.0590 – 0.0717 = - 0.0127	0.0590 – 0.0635 = - 0.0045
16	C18:2(Cis-9, 12)	13.1259 – 13.3723 = - 0.2464	13.1259 – 13.3590 = - 0.2331	13.1259 – 12.7912 = 0.3347	13.1259 – 12.2044 = 0.9215	13.1259 – 13.7937 = - 0.6678
17	C18:2(Trans-9, 11)	0.0434 – 0.0445 = - 0.0011	0.0434 – 0.0305 = 0.0129	0.0434 -0.0456 = - 0.0022	0.0434 – 0.0528 = - 0.0094	0.0434 – 0.0467 = - 0.0033
18	C20:0	0.1030 – 0.1056 = - 0.0026	0.1030 – 0.0724 = 0.0306	0.1030 – 0.1080 = - 0.0050	0.1030 – 0.2461 = -0.1431	0.1030 – 0.1108 = 0.0078
19	C18:3	0.1728 – 0.1771 = - 0.0043	0.1728 – 0.1214 = 0.0514	0.1728 – 0.1812 = - 0.0084	0.1728 – 0.2000 = - 0.00372	0.1728 – 0.1859 = - 0.0131
20	C20:1(Cis-11)	0.1055 – 0.1081 = - 0.0026	0.1055 -0.0741 = 0.0314	0.1055 – 0.1106 = - 0.0051	0.1055 – 0.1282 = - 0.0227	0.1055 – 0.1135 = - 0.0080
21	C18:3(Cis-9, 12, 15)	0.1865 – 0.1911 = - 0.0046	0.1865 – 0.1310 = 0.0555	0.1865 – 0.1955 = - 0.0090	0.1865 – 0.2265 = - 0.0400	0.1865 – 0.2006 = - 0.0141
22	C20:2(Cis-11, 14)	0.0146 – 0.0150 = 0.0004	0.0146 – 0.0102 =0.0044	0.0146 – 0.0153 = - 0.0007	0.0146 – 0.0177 = - 0.0031	0.0146 – 0.0157 = - 0.0011
23	C22:0	0.0950 – 0.0974 = - 0.0024	0.0950 – 0.0668 = 0.0282	0.0950 – 0.0997 = - 0.0047	0.0950 – 0.1155 = - 0.0205	0.0950 – 0.1023 = - 0.0073
24	C20:3(Cis-8, 11, 14)	0.1202 – 0.1232 = - 0.0030	0.1202 – 0.0844 = 0.0358	0.1202 – 0.1261 = - 0.0059	0.1202 – 0.1461 = - 0.0259	0.1202 – 0.1293 = - 0.0091
25	C22:1(Cis-13)	0.0327- 0.0335 = - 0.0008	0.0327- 0.0230 = 0.0097	0.0327- 0.0343 = - 0.0016	0.0327- 0.0397 = - 0.0070	0.0327- 0.0352 = -0.0025
26	C20:3(Cis-11, 14, 17)	0.0630 – 0.0646 = -0.0016	0.0630 – 0.0443 = 0.0187	0.0630 – 0.0661 = - 0.0031	0.0630 – 0.0770 = - 0.0140	0.0630 – 0.0678 = - 0.0048
27	C20:4(Cis-5, 8, 11, 14)	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
28	C22:2(Cis-13, 16)	0.0117 – 0.0120 = -0.0003	0.0117 – 0.0082 = 0.0035	0.0117 – 0.0123 = - 0.0006	0.0117 – 0.0143 = - 0.0026	0.0117 – 0.0126 – 0.0009
29	C24:0	0.0117 – 0.0120 = -0.0003	0.0117 – 0.0082 = 0.0035	0.0117 – 0.0123 = - 0.0006	0.0117 – 0.0143 = - 0.0026	0.0117 – 0.0126 – 0.0009
30	C20:5(Cis-5, 8, 11, 14, 17)	0.0117 – 0.0120 = -0.0003	0.0117 – 0.0082 = 0.0035	0.0117 – 0.0123 = - 0.0006	0.0117 – 0.0143 = - 0.0026	0.0117 – 0.0126 – 0.0009
31	C24:1(Cis-15)	0.0117 – 0.0120 = -0.0003	0.0117 – 0.0082 = 0.0035	0.0117 – 0.0123 = - 0.0006	0.0117 – 0.0143 = - 0.0026	0.0117 – 0.0126 – 0.0009
32	C24:6(Cis-4, 7, 10, 13, 16, 19)	0.0221 – 0.0227 = - 0.0006	0.0221 – 0.0155 = 0.0066	0.0221 – 0.0232 = - 0.0011	0.0221 – 0.0269 = - 0.0045	0.0221 – 0.0126 = 0.00095
	Total (-ve)	- 1.5669	- 1.9692	- 1.4831	- 1.1905	- 2.5693
	Total (+ve)	+ 1.5719	+ 1.9689	+ 1.4826	+ 1.1842	+ 2.5697
	Grand total	**0.0050**	**-0.0003**	**0.0005**	**0.0063**	**0.0004**

Table 3b. Fatty acids content (%) of microbiological degradation products of palm oil (Day 4)

S/N	Fatty Acids	Inoculated oil				
		***Klebsiella* sp. (m)**	***Pseudomonas* sp. (j)**	***Pseudomonas* sp. (n)**	***Bacillus* sp. (p)**	***Staphylococcus* sp. (r)**
1	C5:0	ND – 0.0000 = ND	ND – 0.0000 = ND	ND – 0.0000 = ND	ND – 0.0000 = ND	ND – 0.0000 = ND
2	C6:0	0.0000 – ND = ND	0.0000 – ND = ND	0.0000 – ND = ND	0.0000 – ND = ND	0.0000 – ND = ND
3	C8:0	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
4	C10:0	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
5	C12:0	0.0111 – 0.0058 = 0.0053	0.0111 – 0.0087 = 0.0024	0.0111 – 0.0069 = 0.0042	0.0111 – 0.0116 = - 0.0005	0.0111 – 0.0095 = 0.0016
6	C14:0	0.8346 – 0.8644 = - 0.0298	0.8346 – 0.9162 = - 0.0816	0.8346 – 0.9210 = - 0.0864	0.8346 – 0.8872 = - 0.0526	0.8346 – 0.9495 = 0.1104
7	C14:1(Cis-9)	0.0338 – 0.0175 = - 0.0298	0.0338 – 0.0263 = 0.0075	0.0338 – 0.0208 = 0.0130	0.0338 – 0.0354 = - 0.0016	0.0338 – 0.0287 = 0.0051
8	C16:0	38.9032 – 40.8263 = - 1.9231	38.9032 – 42.3723 = - 3.4691	38.9032 – 42.2744 = - 3.3712	38.9032 – 40.7723 = - 1.8691	38.9032 – 42.6145 = - 3.7113
9	C16:1(Cis-9)	0.1207 – 0.0625 = - 0.0518	0.1207 – 0.0940 = - 0.0833	0.1207 – 0.0743 = - 0.0636	0.1207 – 0.1263 = - 0.1156	0.1207 – 0.1027 = - 0.0920
10	C18:0	5.5831 – 5.7294 = - 0.1463	5.5831 – 6.2206 = - 0.6375	5.5831 – 6.3218 = - 0.7387	5.5831 – 6.0029 = - 0.4198	5.5831 – 6.6556 = - 1.0725
11	C18:1(Trans-6)	0.0518 – 0.0269 = 0.0249	0.0518 – 0.0403 = 0.0115	0.0518 – 0.0319 = 0.0199	0.0518 – 0.0542 = - 0.0024	0.0518 – 0.0441 = 0.0077
12	C18:1(Cis-6)	5.5851 – 4.8334 = 0.7517	5.5851 – 4.2186 = 1.3665	5.5851 – 4.4810 = 1.1041	5.5851 – 4.4066 = 1.1785	5.5851 – 4.2292 = 1.3559
13	C18:1(Trans-9)	0.0034 – 0.0117 = - 0.0083	0.0034 – 0.0026 = 0.0008	0.0034 – 0.0021 =0.0013	0.0034 – 0.0035 = - 0.0001	0.0034 – 0.0029 = 0.0005
14	C18:1(Cis-9)	34.6824 – 34.0712 = 0.6112	34.6824 – 33.3991 = 1.2833	34.6824 – 33.6213 = 1.0611	34.6824 – 34.6824 = 0.1588	34.6824 – 32.7891 = 1.8933
15	C18:1(Trans-11)	0.0590 – 0.0806 = 0.0284	0.0590 – 0.0459 = 0.0131	0.0590 – 0.0363 = 0.0227	0.0590 – 0.0617 = -0.0027	0.0590 – 0.0502 = 0.0088
16	C18:2(Cis-9, 12)	13.1259 – 13.0091 = 0.1168	13.1259 – 11.8724 = 1.2535	13.1259 – 11.5896 1.5362	13.1259 – 12.0628 = 1.0631	13.1259 – 11.6730 = 1.4529
17	C18:2(Trans-9, 11)	0.0434 – 0.0225 = 0.0209	0.0434 – 0.0338 = 0.0096	0.0434 – 0.0267 = 0.0167	0.0434 – 0.0454 = - 0.0020	0.0434 – 0.0370 = 0.0064
18	C20:0	0.1030 – 0.0534 = 0.0496	0.1030 – 0.0802 = 0.0228	0.1030 – 0.0634 = 0.0396	0.1030 – 0.10777 = - 0.0047	0.1030 – 0.0876 = 0.0154
19	C18:3	0.1728 – 0.0896 = 0.0832	0.1728 – 0.1345 = 0.0383	0.1728 – 0.1063 = 0.0665	0.1728 – 0.1807 = - 0.0079	0.1728 – 0.1470 = 0.0258
20	C20:1(Cis-11)	0.1055 – 0.0547 = 0.0508	0.1055 – 0.0821 = 0.0234	0.1055 – 0.0649 = 0.0406	0.1055 – 0.1103 = - 0.0048	0.1055 – 0.0897 = 0.0158
21	C18:3(Cis-9, 12, 15)	0.1865 – 0.0967 = 0.0898	0.1865 – 0.1452 = 0.0413	0.1865 – 0.1147 = 0.0718	0.1865 – 0.1950 = - 0.0085	0.1865 – 0.1586 = 0.0279
22	C20:2(Cis-11, 14)	0.0146 – 0.0076 = 0.0070	0.0146 – 0.0114 = 0.0032	0.0146 – 0.0090 = 0.0056	0.0146 – 0.0153 = - 0.0007	0.0146 – 0.0124 = 0.0022
23	C22:0	0.0950 – 0.0493 = 0.0457	0.0950 – 0.0740 = 0.0210	0.0950 -0.0585 = 0.0365	0.0950 – 0.0994 = - 0.0044	0.0950 – 0.0808 = 0.0142
24	C20:3(Cis-8, 11, 14)	0.1202 – 0.0623 = 0.0579	0.1202 – 0.0936 = 0.0266	0.1202 – 0.0739 = 0.0463	0.1202 – 0.1257 = - 0.0055	0.1202 – 0.1023 = 0.0179
25	C22:1(Cis-13)	0.0327- 0.0169 = 0.0158	0.0327- 0.0254 = 0.0073	0.0327- 0.0201 =0.0126	0.0327- 0.0342 = - 0.0015	0.0327- 0.0278 = =0.0015
26	C20:3(Cis-11, 14, 17)	0.0630 – 0.0327 = 0.0303	0.0630 – 0.0491 = 0.0139	0.0630 – 0.0388 = 0.0242	0.0630 – 0.0659 = - 0.0029	0.0630 – 0.0536 = 0.0094
27	C20:4(Cis-5, 8, 11, 14)	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
28	C22:2(Cis-13, 16)	0.0117 – 0.0061 = 0.0056	0.0117 – 0.0091 = 0.0026	0.0117 – 0.0072 = 0.0045	0.0117 – 0.0123 = - 0.0006	0.0117 – 0.01000 = 0.0017
29	C24:0	0.0117 – 0.0061 = 0.0056	0.0117 – 0.0091 = 0.0026	0.0117 – 0.0072 = 0.0045	0.0117 – 0.0123 = - 0.0006	0.0117 – 0.01000 = 0.0017
30	C20:5(Cis-5, 8, 11, 14, 17)	0.0117 – 0.0061 = 0.0056	0.0117 – 0.0091 = 0.0026	0.0117 – 0.0072 = 0.0045	0.0117 – 0.0123 = - 0.0006	0.0117 – 0.01000 = 0.0017
31	C24:1(Cis-15)	0.0117 – 0.0061 = 0.0056	0.0117 – 0.0091 = 0.0026	0.0117 – 0.0072 = 0.0045	0.0117 – 0.0123 = - 0.0006	0.0117 – 0.01000 = 0.0017
32	C24:6(Cis-4, 7, 10, 13, 16, 19)	0.0221 – 0.0115 = 0.0106	0.0221 – 0.0172 = 0.0049	0.0221 – 0.0136 = 0.0085	0.0221 – 0.0231 = - 0.0010	0.0221 – 0.0188 = 0.0033
	Total (-ve)	- 2.0992	- 4.1882	- 4.1963	- 2.3889	- 4.8942
	Total (+ve)	+ 2.0986	+ 4.1880	+ 4.1959	+ 2.4004	+ 4.8938
	Grand total	**0.0006**	**0.0002**	**0.0004**	**0.0115**	**0.0004**

Table 3c. Fatty acids content (%) of microbiological degradation products of palm oil (Day 7)

S/N	Fatty Acids	Inoculated oil				
		***Klebsiella* sp. (m)**	***Pseudomonas* sp. (j)**	***Pseudomonas* sp. (n)**	***Bacillus* sp. (p)**	***Staphylococcus* sp. (r)**
1	C5:0	ND – 0.0000 = ND	ND – 0.0000 = ND	ND – 0.0000 = ND	ND – 0.0000 = ND	ND – 0.0000 = ND
2	C6:0	0.0000 – ND = ND	0.0000 – ND = ND	0.0000 – ND = ND	0.0000 – ND = ND	0.0000 – ND = ND
3	C8:0	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
4	C10:0	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
5	C12:0	0.0111 – 0.0114 = - 0.0003	0.0111 – 0.0079 = 0.0032	0.0111 – 0.010 = 0.0011	0.0111 – 0.0092 = 0.0019	0.0111 – 0.0926 = - 0.0815
6	C14:0	0.8346 – 1.2528 = 0.4182	0.8346 – 1.7267 = - 0.8921	0.8346 – 1.1934 = - 0.3588	0.8346 – 1.2390 = 0.4014	0.8346 – 2.3149 = - 1.4803
7	C14:1(Cis-9)	0.0338 – 0.0379 = - 0.0041	0.0338 – 0.0239 = 0.0099	0.0338 – 0.0302 = 0.0036	0.0338 – 0.0278 = 0.0060	0.0338 – 0.0281 = 0.0057
8	C16:0	38.9032 – 42.1596 = - 3.2564	38.9032 – 43.9487 = - 5.0455	38.9032 – 43.0051 = - 4.1019	38.9032 – 44.3489 = 5.4457	38.9032 – 44.4150 = - 5.5118
9	C16:1(Cis-9)	0.1207 – 0.1355 = - 0.0148	0.1207 – 0.0853 = 0.0354	0.1207 – 0.1079 = 0.0128	0.1207 – 0.0993 = 0.0214	0.1207 – 0.1003 = 0.0204
10	C18:0	5.5831 – 6.6000 = - 1.01069	5.5831 – 7.6778 = - 2.0947	5.5831 – 6.7753 = - 1.1922	5.5831 – 5.8024 = 0.2193	5.5831 – 9.1367 = - 3.5536
11	C18:1(Trans-6)	0.0518 – 0.0582 = - 0.0064	0.0518 – 0.0366 = 0.0152	0.0518 -0.0463 = 0.0055	0.0518 – 0.0426 = 0.0092	0.0518 – 0.0431 = 0.0087
12	C18:1(Cis-6)	5.5851 – 3.8931 = 1.6920	5.5851 – 3.4299 = 2.1552	5.5851 – 3.9863 = 1.5988	5.5851 – 3.9503 = 1.6348	5.5851 – 3.1174 = 2.4677
13	C18:1(Trans-9)	0.0034 – 0.00038 = - 0.0004	0.0034 – 0.0024 = 0.0010	0.0034 – 0.0030 = 0.0004	0.0034 – 0.0028 = 0.0006	0.0034 – 0.0028 = 0.0006
14	C18:1(Cis-9)	34.6824 – 33.2070 = 1.4754	34.6824 – 31.8857 = 2.7967	34.6824 – 32.8026 = 1.8798	34.6824 – 34.0752 = 0.6072	34.6824 – 31.4083 = 3.2741
15	C18:1(Trans-11)	0.0590 – 0.0662 = - 0.0072	0.0590 – 0.0417 = 0.0173	0.0590 – 0.0528 = 0.0062	0.0590 – 0.0485 = 0.0105	0.0590 – 0.0491 = 0.0099
16	C18:2(Cis-9, 12)	13.1259 – 11.4444 = 1.6815	13.1259 – 10.4223 = 2.7036	13.1259 – 11.0878 = 2.0381	13.1259 – 9.4789 = 3.6470	13.1259 – 8.5392 = 4.5867
17	C18:2(Trans-9, 11)	0.0434 – 0.0488 = - 0.0054	0.0434 – 0.0307 = 0.0127	0.0434 – 0.0388 = 0.0046	0.0434 – 0.0357 = 0.0077	0.0434 – 0.0361 = 0.0073
18	C20:0	0.1030 – 0.1156 = - 0.0126	0.1030 – 0.0728 = 0.0302	0.1030 – 0.0921 = 0.0109	0.1030 – 0.1336 = 0.0306	0.1030 – 0.0856 = 0.0174
19	C18:3	0.1728 – 0.1940 = - 0.0212	0.1728 – 0.1221 = 0.0507	0.1728 – 0.1545 = 0.0183	0.1728 – 0.1420 = 0.0308	0.1728 – 0.1436 = 0.0292
20	C20:1(Cis-11)	0.1055 – 0.1184 = - 0.0129	0.1055 – 0.0746 = 0.0309	0.1055 – 0.0943 = 0.0112	0.1055 – 0.0867 = 0.0188	0.1055 – 0.0877 = 0.0178
21	C18:3(Cis-9, 12, 15)	0.1865 – 0.2093 = - 0.0228	0.1865 – 0.1318 = 0.0547	0.1865 – 0.1667 = 0.0198	0.1865 – 0.1532 = 0.0333	0.1865 – 0.1550 = 0.0315
22	C20:2(Cis-11, 14)	0.0146 – 0.0164 = - 0.0018	0.0146 – 0.0103 = 0.0043	0.0146 – 0.0130 = 0.0016	0.0146 – 0.0120 = 0.0026	0.0146 – 0.0121 = 0.0025
23	C22:0	0.0950 – 0.1067 = - 0.0117	0.0950 – 0.0672 = 0.0278	0.0950 – 0.0850 = 0.0100	0.0950 – 0.0781 = 0.0169	0.0950 – 0.0790 = 0.0610
24	C20:3(Cis-8, 11, 14)	0.1202 – 0.1349 = - 0.0147	0.1202 – 0.0850 = 0.0352	0.1202 – 0.1075 = 0.0127	0.1202 – 0.0988 = 0.0214	0.1202 – 0.0999 = 0.0203
25	C22:1(Cis-13)	0.0327- 0.0367 = - 0.0040	0.0327- 0.0231 = 0.0096	0.0327- 0.0292 = 0.0035	0.0327- 0.0269 = 0.0058	0.0327- 0.0272 = 0.0055
26	C20:3(Cis-11, 14, 17)	0.0630 – 0.0707 = - 0.0077	0.0630 – 0.0445 = 0.0185	0.0630 – 0.0563 = 0.0067	0.0630 – 0.0518 = 0.0112	0.0630 – 0.0524 = 0.0106
27	C20:4(Cis-5, 8, 11, 14)	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
28	C22:2(Cis-13, 16)	0.0117 – 0.0132 = - 0.0015	0.0117 – 0.0083 = 0.0034	0.0117 – 0.0105 = 0.0012	0.0117 – 0.0096 = 0.0021	0.0117 – 0.00098 = 0.0019
29	C24:0	0.0117 – 0.0132 = - 0.0015	0.0117 – 0.0083 = 0.0034	0.0117 – 0.0105 = 0.0012	0.0117 – 0.0096 = 0.0021	0.0117 – 0.00098 = 0.0019
30	C20:5(Cis-5, 8, 11, 14, 17)	0.0117 – 0.0132 = - 0.0015	0.0117 – 0.0083 = 0.0034	0.0117 – 0.0105 = 0.0012	0.0117 – 0.0096 = 0.0021	0.0117 – 0.00098 = 0.0019
31	C24:1(Cis-15)	0.0117 – 0.0132 = - 0.0015	0.0117 – 0.0083 = 0.0034	0.0117 – 0.0105 = 0.0012	0.0117 – 0.0096 = 0.0021	0.0117 – 0.00098 = 0.0019
32	C24:6(Cis-4, 7, 10, 13, 16, 19)	0.0221 – 0.0248 = - 0.0027	0.0221 – 0.0156 = 0.0065	0.0221 – 0.0198 = 0.0023	0.0221 – 0.0182 = 0.0096	0.0221 – 0.0184 = 0.0037
	Total (-ve)	- 1.5569	- 8.0323	- 5.6529	- 6.1000	- 10.63
	Total (+ve)	+ 1.5719	+ 8.0322	+ 5.6527	+ 6.0994	+ 10.59
	Grand total	**- 0.0050**	**0.0001**	**0.0002**	**0.0006**	**0.03**

Table 3d. Fatty acids content (%) of microbiological degradation products of palm oil (Day 9)

S/N	Fatty Acids	Inoculated oil				
		***Klebsiella* sp. (m)**	***Pseudomonas* sp. (j)**	***Pseudomonas* sp. (n)**	***Bacillus* sp. (p)**	***Staphylococcus* sp. (r)**
1	C5:0	-	ND – 0.0000 = ND	-	-	-
2	C6:0	-	0.0000 – ND = ND	-	-	-
3	C8:0	-	0.0000 – 0.0000 = 0.0000	-	-	-
4	C10:0	-	0.0000 – 0.0000 = 0.0000	-	-	-
5	C12:0	-	0.0111 – 0.0166 = - 0.0055	-	-	-
6	C14:0	-	0.8346 – 1.3971 = 0.5625	-	-	-
7	C14:1(Cis-9)	-	0.0338 – 0.0504 = - 0.0166	-	-	-
8	C16:0	-	38.9032 – 43.2327 = - 4.3295	-	-	-
9	C16:1(Cis-9)	-	0.1207 – 0.1800 = - 0.0593	-	-	-
10	C18:0	-	5.5831 – 6.7750 = - 1.1919	-	-	-
11	C18:1(Trans-6)	-	0.0518 – 0.0773 = - 0.0255	-	-	-
12	C18:1(Cis-6)	-	5.5851 – 3.8894 = 1.6967	-	-	-
13	C18:1(Trans-9)	-	0.0034 – 0.0050 = - 0.0016	-	-	-
14	C18:1(Cis-9)	-	34.6824 – 32.0583 = 2.6241	-	-	-
15	C18:1(Trans-11)	-	0.0590 – 0.0880 =- 0.0290	-	-	-
16	C18:2(Cis-9, 12)	-	13.1259 – 10.7305 = 2.3954	-	-	-
17	C18:2(Trans-9, 11)	-	0.0434 – 0.06484 = - 0.0214	-	-	-
18	C20:0	-	0.1030 – 0.1537 = - 0.0507	-	-	-
19	C18:3	-	0.1728 – 0.2578 = - 0.0850	-	-	-
20	C20:1(Cis-11)	-	0.1055 – 0.1574 = - 0.0519	-	-	-
21	C18:3(Cis-9, 12, 15)	-	0.1865 – 0.2782 = - 0.0917	-	-	-
22	C20:2(Cis-11, 14)	-	0.0146 – 0.0218 = - 0.0072	-	-	-
23	C22:0	-	0.0950 – 0.1418 = - 0.0468	-	-	-
24	C20:3(Cis-8, 11, 14)	-	0.1202 – 0.1793 = - 0.0591	-	-	-
25	C22:1(Cis-13)	-	0.0327- 0.0488 = -0.0161	-	-	-
26	C20:3(Cis-11, 14, 17)	-	0.0630 – 0.0940 = - 0.0310	-	-	-
27	C20:4(Cis-5, 8, 11, 14)	-	0.0000 – 0.0000 = 0.0000	-	-	-
28	C22:2(Cis-13, 16)	-	0.0117 – 0.0175 = - 0.0058	-	-	-
29	C24:0	-	0.0117 – 0.0175 = - 0.0058	-	-	-
30	C20:5(Cis-5, 8, 11, 14, 17)	-	0.0117 – 0.0175 = - 0.0058	-	-	-
31	C24:1(Cis-15)	-	0.0117 – 0.0175 = - 0.0058	-	-	-
32	C24:6(Cis-4, 7, 10, 13, 16, 19)	-	0.0221 – 0.0330 = - 0.0109	-	-	-
	Total (-ve)	-	- 6.7164		-	-
	Total (+ve)		+ 6.7162			
	Grand total		**0.0002**			

Table 3e. Fatty acids content (%) of microbiological degradation products of palm oil (Day 12)

S/N	Fatty Acids	Inoculated oil				
		***Klebsiella* sp. (m)**	***Pseudomonas* sp. (j)**	***Pseudomonas* sp. (n)**	***Bacillus* sp. (p)**	***Staphylococcus* sp. (r)**
1	C5:0	ND – 0.0000 = ND	ND – 0.0000 = ND	ND – 0.0000 = ND	ND – 0.0000 = ND	ND – 0.0000 = ND
2	C6:0	0.0000 – ND = ND	0.0000 – ND = ND	0.0000 – ND = ND	0.0000 – ND = ND	0.0000 – ND = ND
3	C8:0	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
4	C10:0	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
5	C12:0	0.0111 – 0.0090 = 0.0021	0.0111 – 0.0103 = 0.0008	0.0111 – 0.0090 = 0.0021	0.0111 – 0.0088 = 0.0023	0.0111 – 0.0096 = 0.0015
6	C14:0	0.8346 – 3.5050 = - 2.6704	0.8346 – 2.2883 = - 1.4537	0.8346 – 2.6413 = - 1.8067	0.8346 – 3.4486 = - 2.614	0.8346 – 3.0817 = - 2.2471
7	C14:1(Cis-9)	0.0338 – 0.0274 = 0.0064	0.0338 – 0.0312 = 0.0026	0.0338 – 0.0273 = 0.0065	0.0338 – 0.0266 = 0.0072	0.0338 – 0.0270 = 0.0048
8	C16:0	38.9032 – 44.9397 = - 6.0365	38.9032 – 43.6616 = - 4.7584	38.9032 – 45.9369 = - 7.0337	38.9032 – 45.1265 = 6.2233	38.9032 – 44.3024 = - 5.3992
9	C16:1(Cis-9)	0.1207 – 0.0980 = - 0.0227	0.1207 – 0.1114 = 0.0093	0.1207 – 0.0976 = 0.0231	0.1207 – 0.0951 = 0.0256	0.1207 – 0.1038 = 0.0169
10	C18:0	5.5831 – 10.9113 = - 5.3282	5.5831 – 8.8155 = - 3.2324	5.5831 – 10.0470 = - 0.4639	5.5831 – 10.7671 = - 5.1840	5.5831 – 10.8762 = - 5.2931
11	C18:1(Trans-6)	0.0518 – 0.0420 = 0.0098	0.0518 – 0.0478 = 0.0004	0.0518 -0.0418 = 0.0100	0.0518 – 0.04080 = 0.0038	0.0518 – 0.0445 = 0.0073
12	C18:1(Cis-6)	5.5851 – 2.1891 = 3.3960	5.5851 – 3.1066 = 2.4785	5.5851 – 2.5424 = 3.0427	5.5851 – 1.9243 = 3.6608	5.5851 – 2.0092 = 3.5759
13	C18:1(Trans-9)	0.0034 – 0.0027 = 0.0007	0.0034 – 0.0031 = 0.0003	0.0034 – 0.0027 = 0.0007	0.0034 – 0.0026 = 0.0008	0.0034 – 0.0029 = 0.0005
14	C18:1(Cis-9)	34.6824 – 29.116 = 5.5658	34.6824 – 31.8335 = 2.8488	34.6824 – 30.0639 = 4.6185	34.6824 – 30.1320 = 4.5504	34.6824 – 30. 7243 = 3.9581
15	C18:1(Trans-11)	0.0590 – 0.0479 = 0.0111	0.0590 – 0.0544 = 0.0046	0.0590 – 7.6507 = 0.0114	0.0590 – 0.0464 = 0.0126	0.0590 – 0.0507 = 0.0083
16	C18:2(Cis-9, 12)	13.1259 – 8.1746 = 4.9513	13.1259 – 9.1088 = 4.0171	13.1259 -7.6507 = 5.4752	13.1259 – 7.5119 = 5.6142	13.1259 – 7.9020 = 5.2239
17	C18:2(Trans-9, 11)	0.0434 – 0.0352 = 0.0082	0.0434 – 0.0401 = 0.0033	0.0434 – 0.0351 = 0.0085	0.0434 – 0.0342 = 0.0092	0.0434 – 0.0373 = 0.0061
18	C20:0	0.1030 – 0.2047 = - 0.1017	0.1030 – 0.0950 = 0.0080	0.1030 – 0.1634 = - 0.0604	0.1030 – 0.1593 = - 0.0563	0.1030 – 0.0885 = 0.0145
19	C18:3	0.1728 – 0.1401 = 0.0327	0.1728 – 0.1593 = 0.0135	0.1728 – 0.1394 = 0.0334	0.1728 – 0.1359 = 0.0369	0.1728 – 0.0906 = 0.0149
20	C20:1(Cis-11)	0.1055 – 0.0855 = 0.0200	0.1055 – 0.0973 = 0.0082	0.1055 – 0.0851 =0.0204	0.1055 – 0.0830 = 0.0225	0.1055 – 0.1601 = 0.0264
21	C18:3(Cis-9, 12, 15)	0.1865 – 0,1512 = 0.0353	0.1865 -0.1719 = 0.0146	0.1865 – 0.1504 = 0.0361	0.1865 – 0.1467 – 0.0398	0.1865 – 0.1601 = 0.0264
22	C20:2(Cis-11, 14)	0.0146 – 0.0118 = 0.0028	0.0146 – 0.0135 = 0.0011	0.0146 – 0.118 = 0.0028	0.0146 -0.0115 = 0.0031	0.0146 – 0.0816 = - 0.0670
23	C22:0	0.0950 -0.0771 = 0.0179	0.0950 – 0.0876 = 0.0074	0.0950 – 0.0767 = 0.0183	0.0950 – 0.0748 = 0.0202	0.0950 – 0.0816 = 0.0134
24	C20:3(Cis-8, 11, 14)	0.1202 – 0.0265 = 0.0937	0.1202 -0.1108 = 0.0094	0.1202 – 0.0970 = 0.0232	0.1202 – 0.0946 = 0.0256	0.1202 – 0.1032 = 0.0170
25	C22:1(Cis-13)	0.0327- 0.0975 = - 0.0648	0.0327- 0.0301 = 0.0026	0.0327- 0.0264 = 0.0063	0.0327- 0.0257 = 0.0070	0.0327- 0.0281 = 0.0046
26	C20:3(Cis-11, 14, 17)	0.0630 – 0.0511 = 0.0119	0.0630 – 0.0581 = 0.0049	0.0630 – 0.0508 – 0.0122	0.0630 – 0.0496 = 0.0134	0.0630 – 0.0541 = 0.0089
27	C20:4(Cis-5, 8, 11, 14)	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000	0.0000 – 0.0000 = 0.0000
28	C22:2(Cis-13, 16)	0.0117 – 0.0095 = 0.0022	0.0117 – 0.0108 = 0.0009	0.0117 – 0.0095 = 0.0022	0.0117 – 0.0092 = 0.0025	0.0117 – 0.0101 = 0.0016
29	C24:0	0.0117 – 0.0095 = 0.0022	0.0117 – 0.0108 = 0.0009	0.0117 – 0.0095 = 0.0022	0.0117 – 0.0092 = 0.0025	0.0117 – 0.0101 = 0.0016
30	C20:5(Cis-5, 8, 11, 14, 17)	0.0117 – 0.0095 = 0.0022	0.0117 – 0.0108 = 0.0009	0.0117 – 0.0095 = 0.0022	0.0117 – 0.0092 = 0.0025	0.0117 – 0.0101 = 0.0016
31	C24:1(Cis-15)	0.0117 – 0.0095 = 0.0022	0.0117 – 0.0108 = 0.0009	0.0117 – 0.0095 = 0.0022	0.0117 – 0.0092 = 0.0025	0.0117 – 0.0101 = 0.0016
32	C24:6(Cis-4, 7, 10, 13, 16, 19)	0.0221 – 0.0179 = 0.0042	0.0221 – 0.0204 = 0.0017	0.0221 – 0.0179 = 0.0042	0.0221 – 0.0175 = 0.0047	0.0221 – 0.0190 = 0.0031
	Total (-ve)	- 14.2016	- 9.4445	- 13. 3647	- 14.0776	- 12.9394
	Total (+ve)	+ 14.2014	+ 9.4443	+ 13.3646	+ 14.0773	+ 12.9394
	Grand total	**0.0002**	**0.0002**	**0.0001**	**0.0003**	**0.0004**

Table 3f. Summary of fatty acid contents (%) of degraded palm oil

Incubation period		Inoculated organisms					Mean	SD	CV%
		KLm	PSj	PSn	BAp	STr			
24hour	Total –ve	1.57	1.97	1.48	1.19	2.57	1.76	0.53	30.4
	Total +ve	1.57	1.97	1.48	1.19	2.57	1.76	0.53	30.4
	Grand total	0.00	0.00	0.00	0.00	0.00	-	-	-
4th day	Total –ve	2.10	4.19	4.19	2.40	4.89	3.55	1.23	34.6
	Total +ve	2.10	4.19	4.19	2.40	4.89	3.55	1.23	34.6
	Grand total	0.00	0.00	0.00	0.00	0.00	-	-	-
7th day	Total –ve	1.57	8.03	5.65	6.10	10.6	6.39	3.33	52.1
	Total +ve	1.57	8.03	5.65	6.10	10.6	6.39	3.33	52.1
	Grand total	0.00	0.00	0.00	0.00	0.00	-	-	-
9th day	Total –ve	-	6.72	-	-	-	-	-	-
	Total +ve	-	6.72	-	-	-	-	-	-
	Grand total	-	0.00	-	-	-	-	-	-
12th day	Total –ve	14.2	9.44	13.4	14.1	12.9	12.8	1.96	15.3
	Total +ve	14.2	9.44	13.4	14.1	12.9	12.8	1.96	15.3
	Grand total	0.00	0.00	0.00	0.00	0.00	-	-	-

Key: KLm- Oil sample inoculated with Klebsiella (m) sp.
PSj- Oil sample inoculated with Pseudomonas (j) sp.
PSn- Oil sample inoculated with Pseudomonas (n) sp.
BAp- Oil sample inoculated with Bacillus (p) sp.
STr- Oil sample inoculated with Staphylococcus (r) sp.

3.3.2 Mineralization of palm oil

The initial mineral contents in the uncooked oil were much higher in concentration than in the cooked oil (Table 4a). Some of these values were high particularly for calcium, sodium and manganese, after preparation of the oil for cooking. The concentrations of co and Mn were extremely low in the extracted oil from wastewater. The most affected minerals in the fresh and the extracted oil from wastewater were manganese, magnesium, potassium and sodium. All these minerals had reduction from fresh to wastewater extracted oil values greater than 99% reduction. the least affected was phosphorus which had a reduction of -5.69% and an average change in calcium with a reduction of -47.8%. It is very likely that *Klebsiella* sp. (m) made use of phosphorus from the onset as its concentration in the oil changed from 9.12 mg/100g to 8.57 mg/100g or 6.03% on the 4th day. This trend also occurred on the 7th day with reduction of 11.2% and on day 12 with reduction of 18.3%. With *Pseudomonas* sp. (j), there was no reduction of phosphorus up to day 4 that is change in value was 0%. However, on day 7 there was reduction from 9.12 mg/100g to 8.99 mg/100g or 1.43% reduction and on the 12th day there was reduction from 9.12 mg/100g to 8.66 mg/100g or 5.04%. This means the reduction in the value of phosphorus could be due to biochemical activities of *Pseudomonas* sp. (j). Phosphorus was only noticed on days 7, 9, and 12 but not on the first day and day 4 as seen in *Klebsiella* sp. (m). Also the biochemical activities of *Klebsiella* sp. (m) appeared to be more drastic on phosphorus when compared to *Pseudomonas* sp. (j). This is because the change in phosphorus value in *Klebsiella spp (m)* was 9.12 mg/100g down to 7.45 mg/100g or 18.3% reduction whereas in the *Pseudomonas* sp. (j), the range of change was 9.12 mg/100g down to 8.32 mg/100g or 8.77% which is less than 18.3% by 52.1%. With *Pseudomonas* sp. (j), there was no change in the value of phosphorus at 24h and day 4 since the value of phosphorus remained 9.12 mg/100g that is 0% change. on the other hand, there was a slight change in day 7, where phosphorus value changed from 9.12 mg/100g to 8.99 mg/100g or 1.43% reduction and in day 12 there was a marginal increase in the biochemical use of phosphorus where it changed from 9.12 mg/100g to 8.66 mg/100g or 5.04%. when considering biodegradation of the oil by *Bacillus* sp. (p) and *Staphylococcus* sp. (r), the phosphorus concentration in the oil remained constant (9.12 mg/100g) for many days; that is a rate change of 0%. in day 4 there was a slight change of 9.12 mg/100g to 9.10 mg/100g or 1.21%. on day 7, phosphorus level change in *Bacillus* sp. (p) was 4.17% reduction whereas *Staphylococcus* sp. (r) for the same day had a reduction of 5.04%, both being lower than *Klebsiella* sp. (m) where the value was reduced by 11.2%. On day 9, no value was recorded for *Staphylococcus* sp. (p) or S*taphylococcus* sp. (r) for all the minerals. On day 12, the reduction of phosphorus in *Bacillus* sp. (p) was 9.32% whereas in *Staphylococcus* sp. (r) it was 8.66% which was respectively lesser than 18.3% of *Klebsiella* sp. (m) by 49.1% and 52.7%. *Klebsiella* sp. (m) utilized about 9.88% calcium (reduction from 7.57 mg/100g to 6.86 mg/100g) after 24h; reduction was 12.5% in day 4; reduction was 16.6% in day 7 and 23.6% in day 12; showing gradual loss of calcium due to biochemical activities of *Klebsiella* sp. (m). However, magnesium reduction in *Klebsiella* sp. (m) degraded oil was maintained at status quo of 33.3% for all the days of observation. it is already seen for magnesium that there was a major reduction between fresh oil and the extracted oil from wastewater (7.51 mg/100g to 0.003 mg/100g), a reduction of 99.96% showing that usually all the mg in the oil had been lost in the cooking process. The use of just 0.001 mg/100g could be assumed to be low for all the days of observation in *Klebsiella* sp. (m).

Pseudomonas sp. (j) used a lower amount of calcium when compared to *Klebsiella* sp. (m), from 7.57 mg/100g to 7.10 mg/100g or reduction of 6.21% (it was 9.38% in *Klebsiella spp (m)*). reduction was slightly higher in day 4 with *Pseudomonas* sp. (j) for calcium with reduction of 7.66%; reduction in day 7 was about 26.0%; it was 30.5% reduction in day 9 and 32.4% in day 12, showing similar degradation of the oil as in *Klebsiella* spp (m). The magnesium levels are similar after 24h and on day 4 in *Pseudomonas* sp. (j), which remained at 0.003 mg/100g or 0% change, meaning that the microbe had no effect on magnesium. In day 7 and 9 there were slight reductions in the magnesium content changing from 0.003 mg/100g to 0.002 mg/100g or reduction of 33.3% for the two different day groups. in the last day 12, the value of magnesium was 0.001 mg/100g which doubled the above percentage at 66.7%. The behaviour of *Pseudomonas* sp. (j) and *Klebsiella* sp. (m) was not similar with the biochemical use of magnesium as seen in the table 4a and b. With *Pseudomonas* sp. (n) the range of calcium change was 6.08% to 22.6%, although no values were reported for day 9. This is not depicting the systematic reduction in the new availability of calcium for biochemical use from 24h to the 12^{th} day. This reduction in calcium is in line with that of *Klebsiella* sp. (m) but less drastic. in magnesium utilization by *Pseudomonas* sp. (n), the behaviour of the microorganism was similar for 24h to day 12 as the value change remained constant from 0.003 mg/100g to 0.002 mg/100g for the days meaning a constant reduction of 33.3% for all the days. This is exactly the observation made in *Klebsiella* sp. (m); meaning that *Klebsiella* sp. (m) and *Pseudomonas* sp. (n) will metabolize magnesium to the same extent.

Bacillus sp. (p) metabolized the oil with calcium concentration being reduced from 6.71 mg/100g to 6.01 mg/100g or 11.4% to 20.6%, although no values were reported in day 9. Unpaired-wise comparison between *Klebsiella* sp. (m) and *Bacillus* sp. (p) showed that a reduction of calcium level at 24h was greater in *Bacillus* sp. (p) than *Klebsiella* sp. (m); on the 4^{th} and 7^{th} days but lower in *Bacillus* sp. (p) than *Klebsiella* sp. (m) on day 12. With *Staphylococcus* sp. (r), the change in calcium utilization was much lower than in the previous observation under *Klebsiella* sp. (m), *Pseudomonas* sp. (j), *Pseudomonas* sp. (n) and *Bacillus* sp. (p), as it ranged from 3.04% to18.9% in reduction. Magnesium change was least at 24h with a change of 0.003 mg/100g to 0.002 mg/100g or 32.3% reduction. After this timeline the rate of change became constant at 66.6% on day 4, day 7 and day 12 but no value was recorded for day 9. in general, *Klebsiella* spp (m) appeared to be the most outstanding in its biochemical use of the minerals; whereas *Staphylococcus* sp. (r) appeared to utilize the minerals minimally. This could be asserted because all other conditions in each medium are uniform. so any change observed can be conveniently said to be due to the microbiological activities on the various minerals.

Table 4a. Mineral values (mg/100g) of degraded palm oil for specific incubation period

Incubation period	Minerals	Fresh uncooked oil	*Cooked oil	Inoculated organisms				
				KLm	PSj	PSn	BAp	STr
24h	Co	<0.001	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	Mn	0.66	<0.001(-99.8%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	Ca	14.5	7.57 (-47.8%)	6.86(9.38%)	7.10(6.21%)	7.11(6.08%)	6.71(11.4%)	7.34(3.04%)
	Mg	7.51	0.003 (-99.96%)	0.002(33.3%)	0.003(0%)	0.002(33.3%)	0.001(66.7%)	0.002(33.3%)
	K	9.57	<0.001(-99.99%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	Na	18.6	<0.001(-99.99%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	P	9.67	9.12 (-5.69%)	8.57(6.03%)	9.12(0%)	9.12(0%)	9.12(0%)	9.12(0%)
Day 4	Co	<0.001	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	Mn	0.66	<0.001 (-99.8%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	Ca	14.5	7.57(-47.8%)	6.62(12.5%)	6.99(7.66%)	6.86(9.38%)	6.58(13.1%)	6.99(7.66%)
	Mg	7.51	0.003(-99.96%)	0.002(33.3%)	0.003(0%)	0.002(33.3%)	0.002(33.3%)	0.001(66.7%)
	K	9.57	<0.001(-99.99%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	Na	18.6	<0.001(-99.99%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	P	9.67	9.12(-5.69%)	8.37(8.22%)	9.12(0%)	9.12(0%)	9.01(1.21%)	8.85(2.96%)
Day 7	Co	<0.001	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	Mn	0.66	<0.001 (-99.8%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	Ca	14.5	7.57(-47.8%)	6.31(16.6%)	5.60(26.0%)	6.22(17.8%)	6.19(18.2%)	6.47(14.5%)
	Mg	7.51	0.003(-99.96%)	0.002(33.3%)	0.002(33.3%)	0.002(33.3%)	0.001(66.7%)	0.001(66.7%)
	K	9.57	<0.001(-99.99%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	Na	18.6	<0.001 (-99.99%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	P	9.67	9.12 (-5.69%)	8.10(11.2%)	8.75(4.06%)	8.99(1.43%)	8.74(4.17%)	8.66(5.04%)
Day 9	Co	<0.001	<0.001(0%)	-	<0.001(0%)	-	-	-
	Mn	0.66	<0.001(-99.8%)	-	<0.001(0%)	-	-	-
	Ca	14.5	7.57(-47.8%)	-	5.26(30.5%)	-	-	-
	Mg	7.51	0.003(-99.96%)	-	0.002(33.3%)	-	-	-
	K	9.57	<0.001(-99.99%)	-	<0.001(0%)	-	-	-
	Na	18.6	<0.001 (-99.99%)	-	<0.001(0%)	-	-	-
	P	9.67	9.12(-5.69%)	-	8.53(6.47%)	-	-	-
Day 12	Co	<0.001	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	Mn	0.66	<0.001(-99.8%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	Ca	14.5	7.57(-47.8%)	5.78(23.6%)	5.12(32.4%)	5.86(22.6%)	6.01(20.6%)	6.14(18.9%)
	Mg	7.51	0.003(-99.96%)	0.002(33.3%)	0.001(66.7%)	0.002(33.3%)	0.001(66.7%)	0.001(66.7%)
	K	9.57	<0.001(-99.99%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	Na	18.6	<0.001 (-99.99%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)	<0.001(0%)
	P	9.67	9.12(-5.69%)	7.45(18.3%)	8.32(8.77%)	8.66(5.04%)	8.27(9.32%)	8.33(8.66%)

*Key: KLm- Oil sample inoculated with Klebsiella (m) sp.; PSj- Oil sample inoculated with Pseudomonas (j) sp.; PSn- Oil sample inoculated with Pseudomonas (n) sp. BAp- Oil sample inoculated with Bacillus (p) sp.; STr- Oil sample inoculated with Staphylococcus (r) sp. = Not detected; *- Cooked oil extracted from wastewater*

Table 4b. Ranges and values of minerals (%) of degraded palm oil after 12 days

Minerals	Range	Value
Cobalt	0%	0%
Manganese	0%	0%
Calcium	3.04 - 18.9%	15.9%
Magnesium	0.00 - 66.7%	66.7%
Potassium	0%	0%
Sodium	0%	0%
Phosphorus	0.00 - 18.3%	18.3%

4. DISCUSSION

4.1 Degradation of oil-rich Wastewater

Few of the isolates exhibited maximum growth after five (5) days of biodegradation of wastewater. However, the strains of *Bacillus* sp. (p) and *Pseudomonas* sp. (j) grew very well (weight > 0.10 mg) after 6 days. On day 12, some isolates still produced twice the initial cell mass after 24h incubation. *Pseudomonas* sp. (dd) recorded over 300% weight increase. This observation is in agreement with the report of Mohd Khairul [11] that microbes which are able to degrade the contaminants increased in numbers when the contaminants are degraded.

The initial weights of the bacterial isolates in palm oil after 24h ranged between 0.33mg and 0.60mg while on the 12^{th} day, the weights ranged between 0.25mg and 0.51mg. Generally, the optimum growth in all the thirty two (32) lipolytic microbial isolates in the fresh oil was between the fifth and seventh days. The growth rate per day ranged between 0.02mg/day in *Pseudomonas* sp. (n), *Klebsiella* sp. (m) 0.03 mg/day, *Pseudomonas* sp. (j) 0.04 mg/day and *Staphylococcus* sp. (r) 0.04 mg/day, *Bacillus* sp. (p) 0.05 mg/day. The decomposition of dietary oil was primarily dependent on the lipolytic ability of the different bacteria. There was more appreciable microbial growth in the wastewater than in the palm oil. This might be due to the presence of some food debris in the wastewater which might have served as ready source of nutrients in spite of the presence of detergent, a source of deleterious substances for the microbes. Meanwhile the non-availability of some essential nutrients in the palm oil made it just suitable medium for minimum/fair microbial proliferation. This observation is in line with the report of Pallavi et al [12] that many bacterial lipases, particularly those from members of the genera *Pseudomonas*, *Bacillus*, *Staphylococcus*, *and Achromobacter* have been frequently associated with wastewater.

The isolation of some strains of *Pseudomonas* and *Staphylococcus* spp. with lipolytic activity in this study also correlates with the findings of Prasad and Manjunath [13] who reported the ability of these organisms to produce highly-efficient lipases which degraded lipid-rich wastewater both individually and when used as a consortium. The decomposition of dietary oil might have been primarily dependent on the lipolytic ability of the various bacteria as was observed by varied cell weight differences. Each organism produced specific and different amounts of lipases which reflected on how the bacterial cells degraded/utilized the oil samples. The report of Dunhaupt et al [14] and others is in support of the findings in this work. Lipase presence not only catalyzed hydrolytic reactions but also catalyzed inter-esterification reactions, depending on the source of lipase and reaction conditions [15;16]. There was variation in the activities of the organisms based on the substrates in which they

grew. There was more appreciable microbial growth in the wastewater as a substrate than in palm oil. Odeyemi *et al*. [16] had earlier reported that the presence of some food debris in wastewater created a favourable condition for the microbes as readily available source of nutrients. Similarly Odeyemi et al [17] noted that the rate of microbial secretions of enzymes was low for the utilization of limited amount of nutrients available in palm oil.

4.2 Degradation of Palm Oil

4.2.1 Biodegradation of minerals

The value of sodium compared to potassium would show that sodium may likely not be more oil soluble than potassium, whereas calcium may likely be more soluble in the edible oil than manganese and phosphorus. This observation confirmed the report of Adeyeye [18] who analyzed the cotton seed (whole meal and defatted meal) with values of sodium being 545.2 ± 5.0 mg/100g (whole meal) and 103.3 ± 5.0 mg/100g (defatted meal) whereas, it was 585.6 ± 9.0 mg/100g (whole meal) and 84.6 ± 3.0 (defatted meal) in potassium; same observation was in calcium, magnesium, iron, manganese etc. Cobalt was low, which is a normal characteristic of the metal from plant sources. The value of manganese at 0.66 mg/100g shows that manganese may be more soluble in oil from plant than from animal source (Table 4a). The reduction of these minerals in cooked oil (extracted from wastewater) could have been brought about by heat during cooking.

The issue of solubility or extraction of the minerals from fresh palm oil could also have resulted into lower values of the minerals in the cooked oil. These further shows the level of minerals now left in the oil from day 1 to day 12 for microbiological use. These are seen under various microbial activities. It is generally noted that for all the days and the inoculated bacteria, the value of cobalt never changed, neither the manganese concentration. Cobalt, manganese, potassium and sodium could be grouped under similar category of less than 0.001 mg/100g under all the days and the microorganisms. The exceptional cases among the minerals were calcium, magnesium and phosphorus for the days and the microorganisms.

4.2.2 Biodegradation of lipids

Note the observation: Initial (undegraded–degraded) value. For example, taking *Klebsiella* sp. (m); C12:0 FA had 0.0111- 0.0114 = - 0.0003. This simply shows that the excess might have come from under-utilized fatty acids that gave positive (+ve) balance under the same condition at the end of the reaction giving a balanced biochemical utilization of fatty acids.

In all the treatments, the grand total of the fatty acids for the negative and positive values, each gave a grand total of virtually 0.00% of the fatty acids content. This indicates that the totality of the microbiological behaviour tends towards the same pattern; the only difference being the way each microorganism had brought out the biochemical utilization of the fatty acids. From Table 3f, it could be deduced that each of the microorganisms can be used as a marker in monitoring the biodegradation of the palm oil fatty acids although at different levels of biodegradation.

5. CONCLUSION

This type of observation will lay a very good foundation in giving the baseline information for this type of work since less attention is paid on degradation of edible oil unlike the type of attention paid to heavy metals in the effluent samples obtained in the environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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