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CHANGES IN THE MICROFLORA AND CHEMICAL COMPONENTS OF DOMESTIC OIL-RICH WASTEWATER

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ABSTRACT

Aerobic and anaerobic changes in the microflora and chemical components of domestic oil-rich wastewater were investigated. Enumeration of total bacterial and coliform counts was determined using spreading method of isolation. Enumeration and characterization of fatty acids were determined using High Performance Liquid Chromatography technique. The microbial load was at its peak on the third day of storage, while the coliforms rose to about 50% and 60% of the total load during aerobic and anaerobic conditions respectively. Anaerobiosis did not affect the total coliform load drastically (47%) on the 12^{th} day of degradation. Among the fatty acids elucidated from fresh dietary oil were lauric (0.99%), myristic (1.00%), palmitic (44.3%). Percentage concentration of fatty acids of extracted oil from aerobic cultured wastewater was as follows: lauric (ND - 5.70%); myristic (ND - 39.5%), palmitic (0.11% - 0.79%), stearic (0.012% - 0.32%), oleic (19.0% - 48.0%) and linoleic (ND - 49.0%), while anaerobic culture produced lauric (ND - 7.3%), myristic (ND - 50.4%), palmitic (0.23% - 0.68%), stearic (0.034% - 0.74%), oleic (13.0% - 52.0%) and linoleic (16.0% - 58.0%) at different storage times. The changes in titratable acidity, mineral and proximate contents with their effects were discussed.

Keywords: Aerobic, anaerobiosis, microflora, fatty acid, wastewater

INTRODUCTION

In Nigeria, very little attention is given to the treatment of wastewater from domestic households such as hotels, restaurants and *bukateria* (**Adeyemo, 2003**) where a large percentage of waste is generated. Some of these wastewater are collected in open landfills or allowed to drain into the open land and uncovered drainages where they eventually flow into nearby stream or river (**Akpan, 2004**), a common incidence especially during the rains. A specific example of what happens is the logging of the contaminated water in the soil. In such situations, oxygen becomes less available as an electron acceptor, prompting denitrifying bacteria to reduce available nitrate into gaseous nitrogen which enters the atmosphere with resultant negative effects (**Madigan et al., 2003**).

Leaching into groundwater is a major part of environmental concern, especially due to the recalcitrant nature of some contaminants (**Lapygina et al., 2002**). Yet another devastating consequence of non-treatment of such wastes is the gradual depletion of biological life and health risks to the body of water. Different methods of waste treatment have been developed for the removal of pathogens and the mineralization of the organic components of sewage prior to discharge (**Boadi and Kuitunen, 2003**). However, in Nigeria, like many developing countries, the discharge of untreated wastewater into the environment is still a problem, despite the establishment and operations of Federal Environmental Protection Agency (FEPA) (**Adeyemo, 2003**).

Domestic wastewater contains approximately 0.2 $g.l^{-1}$ to 0.6 $g.l^{-1}$ of organic matter is contributed by human, kitchen, and cleaning wastes. The domestic wastewater composed of proteins and carbohydrates, smaller amounts of lipids, two other groups of contaminants: the anthropogenic organic chemicals and some microbial pathogens (**Tchobanoglous et al., 1991**). Proteins and urea contribute over 97% of nitrogenous compounds typically found in wastewater.

Previously, some researchers have reported different wastewater contaminants in soil and aquatic environments in different parts of Nigeria (**Nwachukwu et al., 2001; Adeyemo, 2003; Akpan, 2004; Adewoye and Lateef, 2004; Efe, 2005**). Biodegradation of fats and oils in wastewater has a potential role in pollution control. Lipase are serine hydrolases of considerable physiological significance and industrial potential that can catalyze numerous reactions such as hydrolysis, inter-esterification, esterification, alcoholysis and aminolysis (**Jaeger and Eggert, 2002**). *Pseudomonas aeruginosa* LP_{602} (a lipase-producing strain isolated from restaurant wastewater) showed good potential for use in the treatment of wastewater containing high lipid

content (**Dharmsthiti and Kuhasuntisook, 1998**). This paper however reports the bio-degradative activity of the flora associated with wastewater from a local restaurant in Ado-Ekiti.

MATERIAL AND METHODS

Source and collection of wastewater samples

The wastewater samples were collected in sterile utensils and dishes from Falegan restaurant, along the Ekiti State Secretariat road, Ado-Ekiti, Nigeria. The restaurant which accommodates about fifty five (55) guests at a sitting opens around 10am and closes by 4pm daily. The main dishes served are rice and beans (cowpea), soup/stew and fried fish, poultry or meat. Other dishes include pounded yam (*iyan*), '*gari', 'amala'* and *'fufu'* served with stew, '*egusi',* spiced vegetable sauce or okra soup as preferred by consumers.

Wastewater samples collected after dish washing comprise food remnants (waste from above food items), cleaning soap solution (detergent and water: 15.6mg $OMO^{*}ml^{-1}$). These samples were collected using 250ml-sized sterile sampling bottles and immediately kept at $25^{o}C \pm 1^{o}C$ in the Microbiology Laboratory of the Ekiti State University, Ado-Ekiti until when needed.

Enumeration of Total Bacterial and Coliform Counts

Wastewater was collected in large bowls (50 litre container) and stored for 12 days under aerobic and anaerobic conditions for the enumeration of total bacteria and coliform counts (These conditions simulate situations where wastewater was disposed into open fields and sealed septic tanks respectively). Wastewater samples were serially diluted and twenty milliliter of molten nutrient agar was cooled to $45^{0}C$ and poured into each Petri-dish to solidify and later inoculated with 1ml of the wastewater sample. The plates were incubated at $37^{0}C$ aerobically and anaerobically (using anaerobic jar) respectively. After 24h incubation, the colonies were counted using colony counter. Total coliform count was also determined on MacConkey agar as described by **Barrow and Feltham (1993)**.

Characterization and Identification of Isolates

The isolates were classified on the basis of biochemical, physiological and morphological characteristics as described by **Olutiola et al. (1991) and Cheesebrough (2006)** and matched against standard microbial cultures.

Physicochemical analyses

About 5ml of concentrated HCl was added to 250ml of wastewater sample and evaporated to 25ml. The concentrate was transferred to a 50cm^3 standard flask and diluted to the mark with distilled de-ionized water (Parker, 1972). The pH was measured with a KENT EIL 7020 pH-meter (Kent Industrial Measurement Limited, Surrey, England) while turbidity was determined with a colorimeter (MODEL 6025, JENWAY, UK) at wavelength of 470nm (**Okonkwo et al. 2008**). The total titratable acidity was measured using the volumetric method.

Mineral Analyses

Zinc, iron, copper, cobalt, lead, manganese, magnesium and calcium were analyzed in triplicates using an Atomic Absorption Spectrophotometer (PYE Unicam Sp 9, Cambridge, UK). Potassium and sodium were analyzed from the sample solutions by using a Flame Photometer (BUCK 2010 VGP AAS) (**AOAC, 2005**).

Proximate Analyses

The proximate component of wastewater samples obtained from different collection sites was analyzed by the method of **Association of Official Analytical Chemists (2005)**. The samples were analyzed for fat, fibre, protein, ash, carbohydrate (glucose) and moisture content. The active ingredients (silica, soda ash, surfactant and phosphate) of the detergent (*OMO*) used in dish washing, were also determined with a Spectronic 20 colorimeter (Gallenkamp, UK) as described by **AOAC (1990)**.

Extraction and Methylation of Oil in the Wastewater Samples

Oil was extracted from 20ml wastewater samples by adding 20ml of concentrated hydrochloric acid, diethyl ether and normal-hexane (1:1:1;v/v/v) (**APHA, 1989**). The resulting mixture was thoroughly shaken and allowed to stand for 30mins in a separating funnel. The organic solvent layer was separated and removed from the oil at ambient temperature.

The extracted oil from the wastewater samples was measured into a test tube and 4ml of 0.5M methanolic sodium hydroxide (20g of NaOH in 1dm^3 methanol) was added and heated on a steam bath for 5mins until it globulised into solution. Five millilitre of BF_3/MeOH (14% Borontrifluoride, 86% Methanol, 100 ml) was added into the test tube and the mixture was boiled for 2min. This mixture was then transferred into a 250ml separating funnel and 30ml of petroleum ether (40 - 60^{o}C boiling range) was added. Twenty millilitre of saturated sodium chloride solution was added and shaken vigorously. The lower layer of the aqueous methanol solution was allowed

to separate. This was drained off and discarded. The petroleum ether layer was then filtered through Whatman No.1 filter paper into a 50ml-sized beaker. The solvent was reduced to a final volume of 10ml. The methyl esters were used for HPLC analysis (**AOAC, 2005**).

Characterization and Enumeration of Fatty Acids in the Methylated Esters

The AKTA basic 10/100 High Performance Liquid Chromatography (HPLC) with serial number 90042 (Amersham Pharmacia Biotech, Sweden) was used for the determination of fatty acids. The analysis was carried out using Supercosil LC-18 column, 25 cm x 3.0 mm ID, and 5 µm particles. The mobile phase consisted of acetonitrile: acetone (59:41, v/v). The mobile phase flow rate was 1.5ml/min. The fatty acids were detected using a UV (UV-215nm) detector. A 20µl sample was injected for each run (**AOAC, 2005**). The fatty acid components in the test samples were identified by comparing the retention times to those of the standards. Each fatty acid in the test sample was expressed as a percentage of the total fatty acids present. The methyl esters of lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids were used as standards for identification of the fatty acids (**APHA, 1989**).

RESULTS AND DISCUSSION

The turbidity values for anaerobic and aerobic conditions ranged between 1.50 to 1.73 and 1.52 to 1.74 respectively. The pH values ranged between 8.80 to 10.4 and 8.92 to 10.3 respectively while the total titratable acidity (TTA) of wastewater samples incubated under anaerobic and aerobic conditions for 12days ranged from 5.00 to 10.00 and 4.00 to 8.33 respectively (Table 1). The pH values decreased gradually under both aerobic (from 10.3 to 8.12) and anaerobic (from 10.4 to 8.69) conditions but there was a remarkable reduction in the pH values on the fifth day (aerobic: 10.3 to 8.92; anaerobic: 10.4 to 8.80) due to increase in biodegradation in the wastewater culture. The acidity resulted from the wastewater culture which is an acidic effluent highly charged with organic matter (**Sheryl et al., 1994**).

Microorganisms in septic systems fill their energy needs by catalyzing the oxidation of organic compounds containing reduced carbon and nitrogen which requires the concurrent reduction of electron acceptors (**Sheryl et al., 1994**). The fairly high alkalinity and near-neutral pH of wastewater are often altered by these redox reactions (**Sheryl et al., 1994**). In poorly aerated systems the degree of aerobic oxidation is lower than in well aerated systems. However, anaerobic digestion may cause substantial CO_2 increases, which may lower pH of the effluent or

dissolve $CaCO_3$. Furthermore, the high moisture contents associated with poorly aerated conditions may hinder CO_2 diffusion to the atmosphere.

Tab 1 Turbidity, pH and total titratable acidity of soap solution, wastewater during storage

	Turbidity*		**pH**		**Total titratable acidity (%)**	
Soap solution	0.002		9.37		0.86	
Fresh wastewater	1.71		11.30		8.33	
Storage (Days)	**Incubation Condition**					
	Aerobic	**Anaerobic**	**Aerobic**	**Anaerobic**	**Aerobic**	**Anaerobic**
1	1.72	1.73	10.27	10.40	7.69	10.00
2	1.68	1.72	9.56	8.69	5.00	9.09
3	1.52	1.50	9.89	9.43	8.33	9.09
4	1.65	1.66	9.45	8.86	8.33	9.09
5	1.71	1.70	8.92	8.80	5.00	7.33
6	1.70	1.68	9.45	9.12	5.00	7.20
7	1.73	1.68	9.69	9.09	4.00	5.00
8	1.74	1.73	9.55	9.21	4.00	5.00
9	1.68	1.69	9.28	9.54	4.00	5.00
10	1.72	1.73	9.13	9.71	4.00	5.00
11	1.72	1.71	9.15	9.72	4.00	5.00
12	1.71	1.71	9.14	9.71	4.00	5.00

Legend: *Colorimeter reading at 470nm

There was decrease in the total titratable acidity values from 8.33 to 4.00 for the aerobic culture with constant value till the twelfth day. The total titratable acidity values increased from 8.33 (fresh sample) to 10.0 on the first day of incubation for anaerobic culture, but reduced gradually on the second day of incubation with constant values as from the seventh day due to reduction in the microflora of the culture. **Sheryl et al. (1994)** stated that most large organic particles are removed in the septic tank by microbes where nitrogen is released as the reduced inorganic ammonium ion (NH_4^+) which leads to increase in total titratable acidity under anaerobiosis.

The coliforms constituted only 5.4% of the total microbial load in the fresh oil-rich wastewater sample. The total bacteria load (TB) was usually higher under aerobic condition while the coliform counts were greater under anaerobiosis (Fig.1).

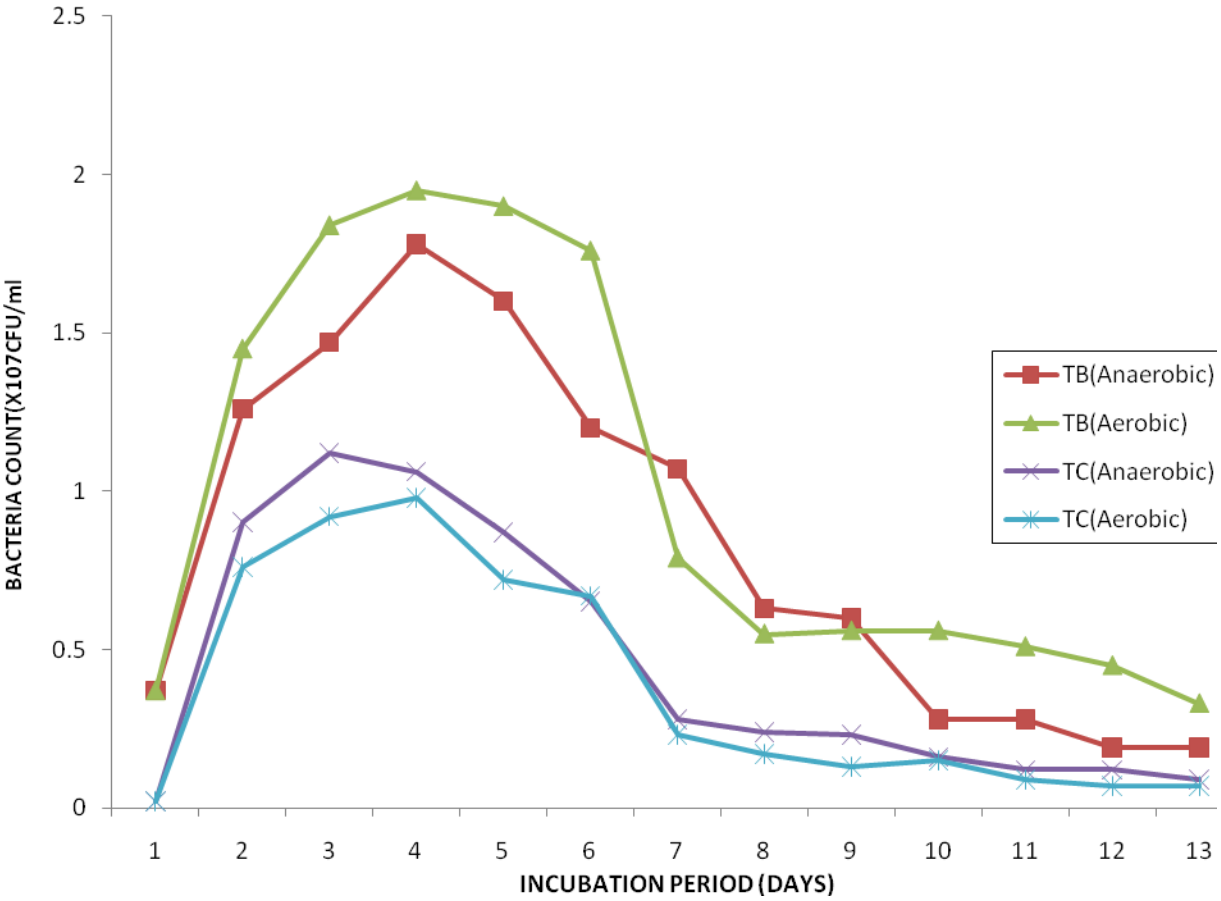

Fig 1 Total Bacteria (TB) and Total Coliform (TC) counts of wastewater samples under anaerobic and aerobic conditions

Bacteria may exist as aerobic, anaerobic or facultative organisms in wastewater based on the available oxygen (BOD) for the degradation of organic matter (**Messner et al., 2006**). **Madigan et al. (2001)** reported the group of organisms commonly encounter in wastewater to include faecal coliform; a group of bacteria commonly associated with human excretion. They are considered to play important roles in biological treatment of wastewaster.

In all cases, the microbial load was at its peak on the third day of storage, where the coliforms rose to about 50% and 60% of the total load during aerobic and anaerobic conditions respectively. This results from the presence of food debris that acts as suitable substrate for the microbes. Anaerobiosis did not affect the total coliform load drastically (47%) on the 12th day of degradation. However, this load was seriously affected under aerobiosis (21%). **George et al. (2003)** reported that aerobic and facultative bacteria majorly oxidize the organic matter in

wastewater to stable and unobjectionable end products like methane, carbon dioxide and ammonia, which eventually encourage anaerobiosis as a prevailing condition in the degradation of wastewater. The high microbial load, especially the coliform, can therefore be attributed to the presence of suspended/particulate solids (**Tijani et al., 2005**).

Altogether, forty seven (47) bacteria isolates were obtained from the wastewater samples under aerobic condition. The isolates were from the following genera: *Escherichia* spp. (3), *Klebsiella* spp. (4), *Staphylococcus* spp. (9), *Shigella* spp. (17), *Enterococcus* spp. (12) and *Salmonella* spp. (2). Meanwhile 63 isolates obtained from the different wastewater samples under anaerobic condition include *Escherichia* spp. (6), *Klebsiella* spp. (5), *Staphylococcus* spp. (5), *Shigella* spp. (19), *Enterococcus* spp. (20) and *Salmonella* spp. (8) making a total of 63 isolates (Table 2). The isolates of the following Genera: *Escherichia*, *Shigella*, *Enterococcus* and *Salmonella* increased in number (25.4%) appreciably under anaerobic condition which is in agreement with **Madigan et al. (2001)** that, the best groups of organisms known in wastewater are the fecal coliforms; a group of bacteria commonly associated with human excretion. **Parker and Mee (1982)** also reported that, in the septic tank the steady input of wastewater provide enough water which is always adequate for pathogen survival. In general, the subsurface fate of wastewater from septic systems has been studied constituent by constituent and most changes in wastewater (most especially the fluctuations in microflora counts of the cultures) occur as a result of the reactions of a few major components that strongly affect the master variables of the system: the redox level and pH (**Reneau et al., 1989**).

Tab 2 Frequency of bacteria isolates from wastewater culture during storage

Storage period (days)	Aerobic							Anaerobic						
	E. coli	*Klebsiella*	*Staphylococcus*	*Shigella*	*Enterococcus*	*Salmonella*	**Total**	*E. coli*	*Klebsiella*	*Staphylococcus*	*Shigella*	*Enterococcus*	*Salmonella*	**Total**
1	-	1	-	2	-	-	3(6.4%)	-	-	-	1	2	-	3(4.8%)
2	1	-	1	-	1	1	4(8.5%)	2	1	1	2	-	2	8(12.7%)
3	-	-	-	1	-	-	1(2.1%)	1	1	-	-	2	-	4(6.3%)
4	1	1	1	2	2	-	7(14.9%)	1	1	1	1	1	1	6(9.5%)
5	-	1	1	2	1	-	5(10.6%)	1	-	-	1	3	-	5(7.9%)
6	-	-	1	2	2	-	5(10.6%)	-	-	-	2	2	2	6(9.5%)
7	-	-	1	2	2	-	5(10.6%)	-	1	-	2	2	1	6(9.5%)
8	1	1	2	-	1	-	5(10.6%)	-	-	-	3	-	1	4(6.3%)
9	-	-	1	-	2	-	3(6.4%)	1	-	-	3	2	-	6(9.5%)
10	-	-	1	2	-	1	4(8.5%)	-	1	3	2	1	-	7(11.1%)
11	-	-	-	1	-	-	1(2.1%)	-	-	-	1	1	-	2(3.2%)
12	-	-	-	3	1	-	4(8.5%)	-	-	-	1	4	1	6(9.5%)
Total	3	4	9	17	12	2	47(100%)	6	5	5	19	20	8	63(100%)

The rate of degradation of oil into fatty acids almost corresponded to the rate of microbial growth for about 7days (Fig 2). The isolation of lipase producing microorganisms capable of degrading fat and oil in wastewater and their degradable efficiency in both single culture and mixed culture formula have been studied (**Bhumibhamon et al., 2002**). The results of degradation of fat and oil between mixed cultures and single culture in two experiments indicated that the very different degradation efficiency might be due to the different reaction system of lipase from each culture. Lipase present not only catalyzed hydrolysis reaction but also catalyzed interesterification reaction, depending on the source of lipase and reaction condition (**Macrae, 1983**). Lipase produced by different organisms such as *Pseudomonas cepacia*, might have different reaction (**Dunhaupt et al., 1992**).

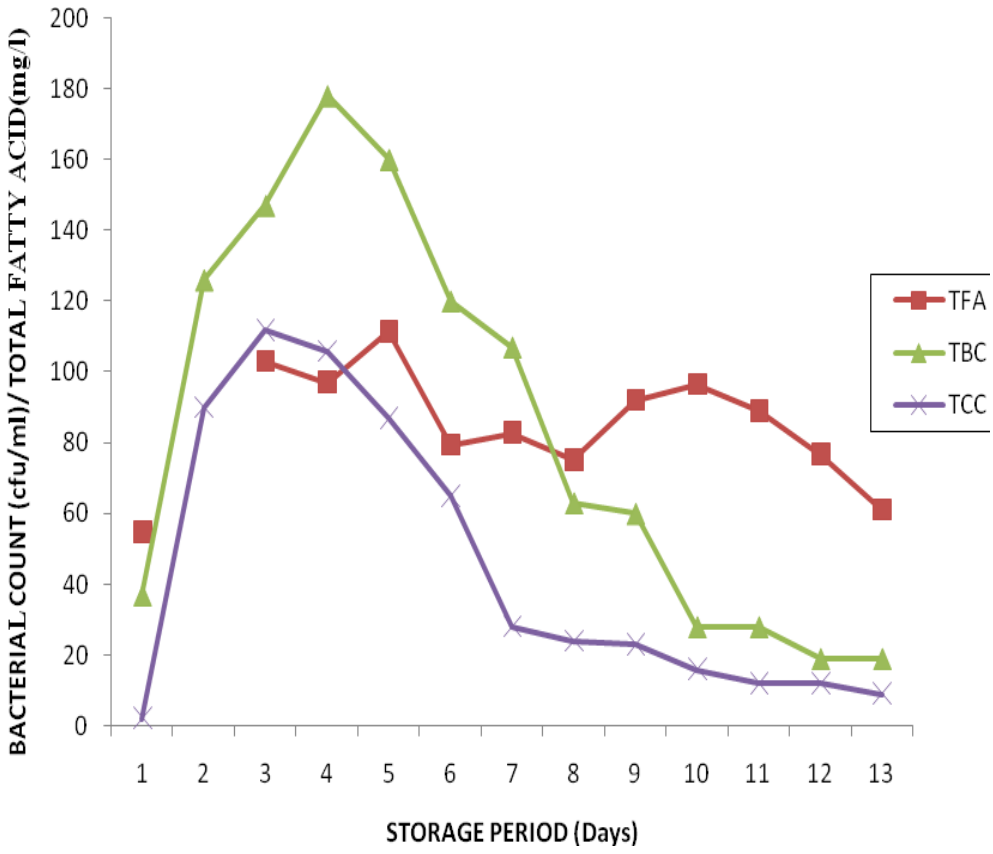

Fig 2 Total Bacterial Count (TBC), Total Coliform Count (TCC) (x 10^5 CFU/ml) and Total Fatty Acid (TFA) concentration under anaerobic conditions

There was initial increase in bacterial growth due to utilization of the available nutrient and secretion of lipases by microbial utilization/degradation of fat and oil in the wastewater

culture. However, there was an inverse proportional relationship in the production of fatty acids to the microbial load after 7days under anaerobiosis. A similar trend was observed under aerobic storage for the first 5days (Fig.3).

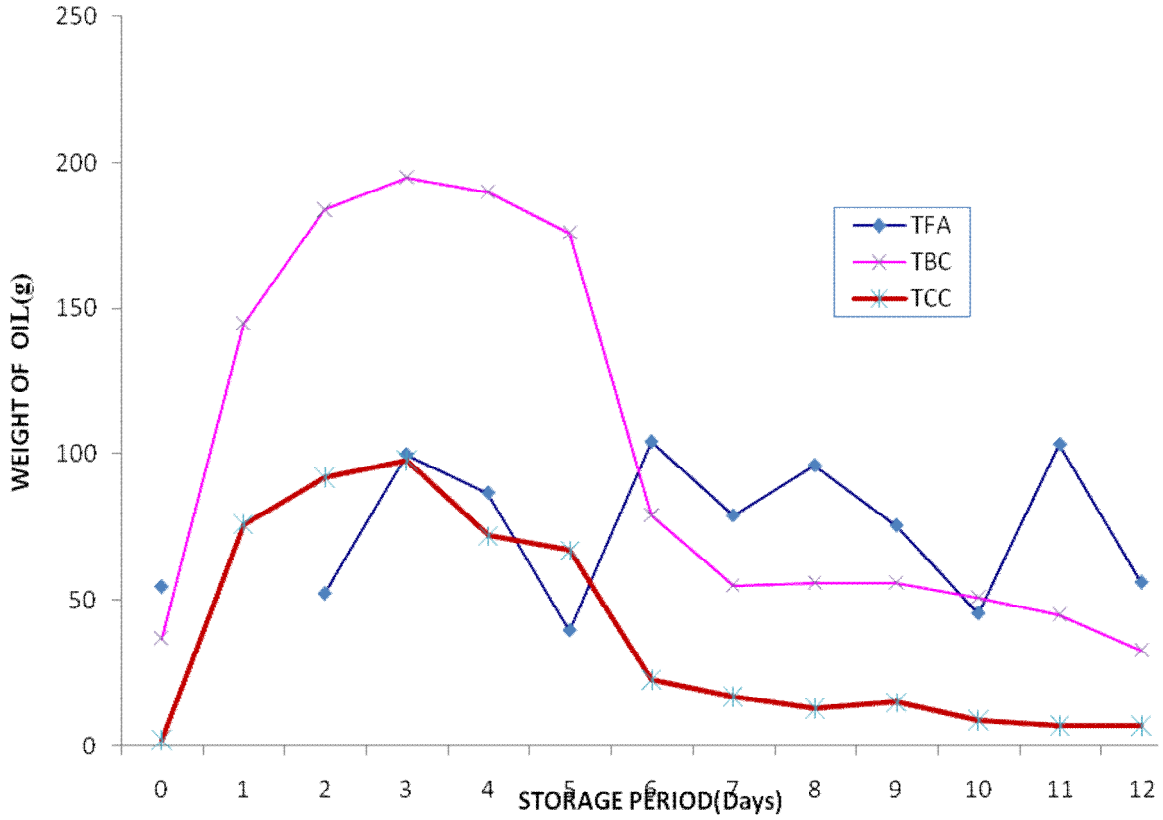

Fig 3 Total Bacterial Count (TBC), Total Coliform Count (TC) (x10^5 CFU/ml) and Total Fatty Acid (TFA) concentration under aerobic conditions

Fatty acid production was usually greater under aerobic biodegradation. **Bhumibhamon et al. (2002)** reported that aerobic lipase producing bacteria are the most appropriate isolates for high fat and oil wastewater treatment.

The fatty acids in the fresh dietary oil are lauric (0.99%), myristic (1.00%), palmitic (44.3%), stearic (4.60%), oleic (38.7%) and linoleic (10.5%). However, the percentage concentration of fatty acids in the extracted oil from wastewater stored under aerobic condition was as follows: lauric (Not Detected - 5.70%); myristic (ND - 39.5%), palmitic (0.11% - 0.79%), stearic (0.012% - 0.32%), oleic (19.0% - 48.0%) and linoleic (ND - 49.0%), while the samples cultured under anaerobic condition produced lauric (ND-7.3%), myristic (ND-50.4%), palmitic (0.23%- 0.68%), stearic (0.034%-0.74%), oleic (13.0%-52.0%) and linoleic (16.0%-58.0%) at different storage times (Table 3). Some of the fatty acids in the extracted oil were not detected during incubation. This might be because of microbial utilization or bio-conversion of some of the

fatty acids. The rate/trend with which the total fatty acids were produced over the storage period was proportional to the microbial counts (Fig. 2 and 3 above). There is synthesis of myristic acid on the second day of aerobic and anaerobic incubations due to bioconversion process. **Moody et al. (1971)** also reported that there was an increase in the amount of longer chain fatty acid (e.g myristic acid) when shorter chain fatty acids such as oleic acid was subjected to heat. Also the concentration of oleic acid was high for both aerobic and anaerobic incubations at ambient temperature. This revealed there was no utilization or bio-conversion of oleic acid by the microbial activities for both aerobic and anaerobic conditions.

Tab 3 Percentage concentration of fatty acids in oil extracted from wastewater culture during storage

Incubation Period (Days)	Aerobic							Anaerobic						
	Lauric acid C12:0	**Myristic acid** C14:0	**Palmitic acid** C16:0	**Stearic acid** C18:0	**Oleic acid** C18:1	**Linoleic acid** C18:2	**Total fatty acid**	**Lauric acid** C12:0	**Myristic acid** C14:0	**Palmitic acid** C16:0	**Stearic acid** C18:0	**Oleic acid** C18:1	**Linoleic acid** C18:2	**Total fatty Acid**
Fresh oil	0.99	1.00	44.3	4.60	38.7	10.5	100.1	0.99	1.00	44.3	4.60	38.7	10.5	100.1
Fresh Wastewater	3.84	ND	0.62	0.37	65.9	29.3	100.03	3.84	ND	0.62	0.37	65.9	29.8	100.3
1	ND	15.6	0.21	0.02	84.1	ND	99.9	3.60	49.1	1.17	0.72	18.6	26.8	100
2	ND	20.7	0.41	0.18	29.9	48.7	99.9	7.51	38.0	0.70	0.36	24.7	28.7	100
3	ND	24.4	0.49	0.22	41.4	33.5	100	ND	23.9	0.50	0.22	33.5	41.9	100
4	ND	21.1	0.44	0.19	78.3	ND	100	4.68	ND	0.63	0.29	51.9	42.5	100
5	ND	22.6	0.45	0.18	37.7	39.0	99.9	3.85	ND	0.52	0.23	63.2	32.2	100
6	5.44	36.2	0.75	0.32	24.0	33.2	99.9	8.97	24.3	0.31	0.04	27.5	38.9	100
7	2.79	41.0	0.77	0.29	28.6	26.6	100	4.68	25.1	0.53	0.23	34.4	35.0	100
8	7.42	ND	0.90	0.04	37.5	54.2	100	ND	21.5	0.49	0.27	45.7	32.0	100
9	8.40	ND	1.16	0.55	43.3	46.6	100	5.87	26.9	0.54	0.21	25.9	40.5	100
10	ND	30.5	0.56	0.20	46.7	22.0	100	6.28	ND	0.66	0.28	16.7	76.0	100
11	7.31	ND	1.06	0.57	46.3	44.8	100	5.57	ND	0.76	0.33	60.6	32.7	100

Legend: ND= Not detected

The weight of extracted oil from wastewater samples stored under anaerobic and aerobic conditions ranged from 1.06 to 2.42g and 1.02 to 2.42g respectively (Figure 4). There was successive reduction in the weight of the extracted oil over the period of 12 days under both aerobic and anaerobic conditions, due to regular utilization of the oil content as source of substrate for the organisms.

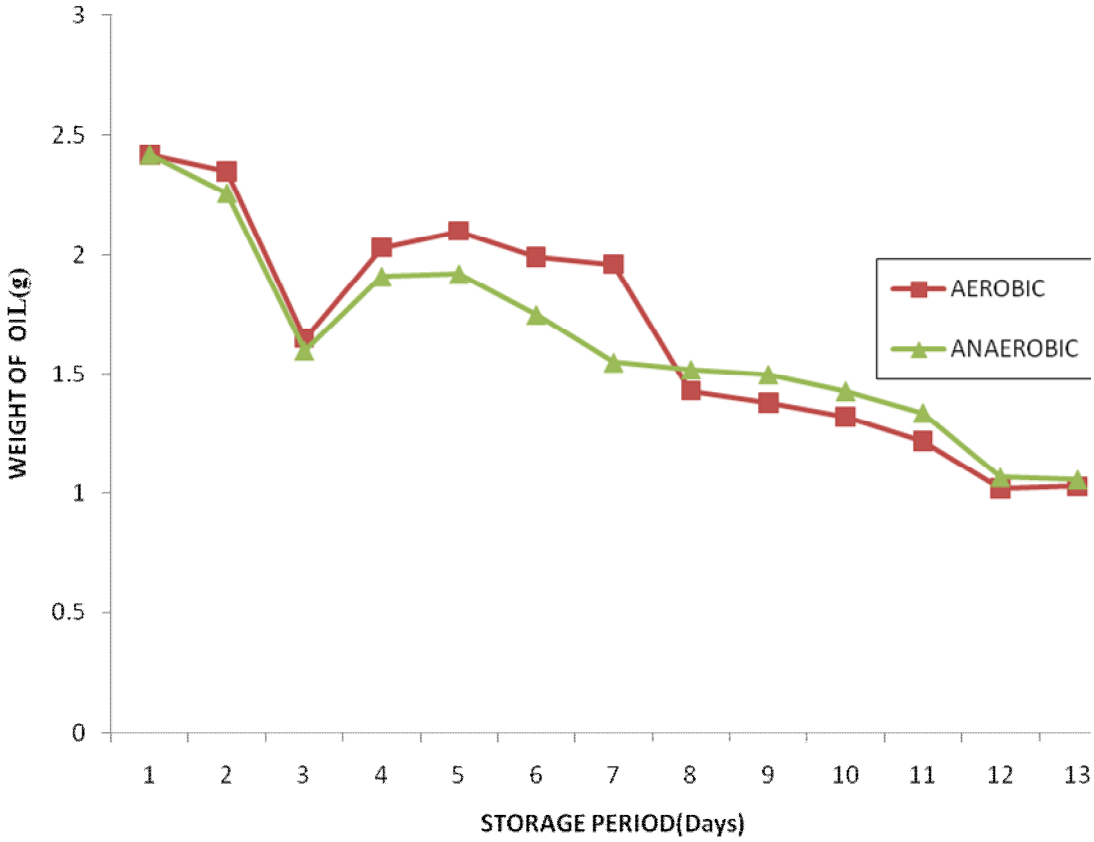

Fig 4 Weight of biodegraded oil (g) during Aerobic and Anaerobic storage

Potassium decreased until the 7th day but there was increase from 49.8 $mg.l^{-1}$ to 51.7 $mg.l^{-1}$ on the third day of incubation. Sodium reduced during storage but with a slight (37.5 $mg.l^{-1}$) on the fourth day of storage. Magnesium increased in value (41.8 $mg.l^{-1}$ or 43.7% increase) on the fourth day while the values of manganese over the period of 12 days were drastically reduced. Zinc reduced during incubation but on the ninth day there was an increase (0.25 $mg.l^{-1}$ or 80% increase). Iron reduced in value but there was increase (35.2 $mg.l^{-1}$ or 24.1% increase) on the sixth day. Calcium also reduced in value but increased to 130.0 $mg.l^{-1}$ on the eighth day (Table 4). This observation is supported by **Sheryl et al. (1994)** who stated that, $CaCO_3$ is often dissolved in drain field in order to buffer the acidity released during NH_4^+ oxidation, which results in increased

Ca^{2+} concentrations in the effluent. Other cations may also be released from the solid phase during buffering reactions such as in mineral dissolution or cation exchange (**Bohn et al., 1985**).

Tab 4 Mineral components ($mg.l^{-1}$) of wastewater cultured under aerobic storage condition

	K	**Na**	**Mg**	**Mn**	**Pb**	**Zn**	**Fe**	**Ca**
Soap solution	1.26	0.95	2.54	ND	ND	1.26	ND	2.54
Fresh oil Incubation period	9.57	18.6	7.51	0.66	ND	9.57	2.63	14.5
Fresh Wastewater	49.8	38.4	50.0	0.20	0.10	0.30	50.2	15.0
1	35.1	35.2	45.1	0.20	ND	0.30	31.0	11.0
2	36.4	31.3	43.1	0.17	0.01	0.10	21.5	11.5
3	51.7	21.1	35.7	0.17	ND	0.16	11.6	11.7
4	32.7	37.5	41.8	0.18	ND	0.09	15.7	7.5
5	28.9	21.7	32.1	0.16	ND	0.10	26.7	11.0
6	19.1	30.6	31.6	0.19	ND	0.08	35.2	11.2
7	17.1	15.9	20.1	0.13	0.01	0.07	19.8	11.9
8	21.5	15.7	25.2	0.09	ND	0.05	17.9	13.0
9	24.6	11.8	19.7	0.10	0.01	0.25	12.7	ND
10	20.7	23.3	27.1	0.13	ND	0.20	21.9	12.6
11	11.5	21.8	17.2	0.12	ND	0.09	25.5	11.7
12	9.70	25.4	21.3	0.08	ND	0.10	15.4	11.0

Legend: ND = Not detected; No traces of Cu were detected in all the samples

Lead was detected initially from the fresh samples and no longer detected in subsequent days of storage. Table 5 showed the mineral values for wastewater incubated anaerobically. There was initial reduction of potassium but there were increases on the sixth and eleventh days (33.3 $mg.l^{-1}$ and 21.1 $mg.l^{-1}$) respectively. There was sequential reduction of sodium during storage. Magnesium reduced from 50.0 to 21.5 $mg.l^{-1}$. Similarly, Zinc reduced during storage but a peak (0.25 $mg.l^{-1}$) was observed on the ninth day. The values of iron were reduced gradually but there were peaks on the fifth (21.7 $mg.l^{-1}$) and ninth (22.5 $mg.l^{-1}$) days. This could be described better

with the phenomenon of oxidation/reduction processes which indirectly affected the pH of the wastewater culture and subsequently increased or reduced the iron content.

Tab 5 Mineral components ($mg.l^{-1}$) of wastewater cultured under anaerobic storage condition

	K	Na	Mg	Mn	Pb	Zn	Fe	Ca
Soap solution	1.26	0.95	2.54	ND	ND	1.26	ND	2.54
Fresh oil	9.57	18.6	7.51	0.66	ND	9.57	2.63	14.5
Incubation period								
Fresh Wastewater	49.8	38.4	50.0	0.20	0.10	0.30	50.2	150
1	21.2	31.5	44.7	0.20	0.10	0.15	41.3	115.1
2	19.3	25.1	48.8	0.08	0.08	0.11	41.6	113
3	18.1	27.3	26.7	0.08	ND	0.11	19.1	75
4	19.3	17.4	21.5	0.08	ND	0.14	19.5	35
5	13.5	17.5	36.7	0.07	ND	0.11	21.7	31
6	33.3	21.5	18.1	0.06	ND	0.07	16.3	21.7
7	25.9	19.2	19.2	0.02	ND	0.03	12.1	72.0
8	30.0	27.1	24.4	ND	0.01	0.10	11.8	48.2
9	17.3	28.1	11.3	ND	0.01	0.25	22.5	45.1
10	18.2	19.7	15.3	0.01	ND	0.01	10.0	114.0
11	21.1	19.5	15.7	0.01	ND	0.01	11.2	19.0
12	17.1	11.8	14.3	ND	ND	0.02	2.20	24.0

Legend: ND = Not detected; No traces of Cu were detected in all the samples

There was reduction in lead within 48h storage; thereafter subsequent wastewater samples did not show any appreciable level of lead. This was due to bioaccumulation of lead in the microbial cell in the culture. Initially there was a sharp fall in the values of calcium; meanwhile there was an increase on the seventh (72.0 $mg.l^{-1}$) and tenth (114.0 $mg.l^{-1}$) days. Lead was detected in the fresh and oil extracted from 24 and 48h stored wastewater samples which may be due to anthropogenic contamination during oil production processes (**Sharif et al., 2008**). The amount of lead on days 8 and 9 was very low (0.01 $mg.l^{-1}$) and the mineral was not detected thereafter. This was due to the bioaccumulation of lead in the microbial cell in the culture. **Malik (2003)** reported the use of lively metal-resistant bacterial/fungal strains as a more effective way of removing metal contaminants.Table 6 showed other parameters analyzed on the wastewater samples cultured aerobically and anaerobically over a period of 12 days. With oil extracted from wastewater stored under aerobic condition, silica reduced initially but increased on the third day (2.20%). Soda ash increased initially but reduced to 1.05% subsequently on the third day. This indicated that, there was degradation of soda ash on the third day.

Tab 6 Silica, soda ash (%), surfactant and phosphate contents ($mg.l^{-1}$) of soap solution, fresh oil and wastewater during storage

	Aerobic				Anaerobic			
	Silica	**Soda ash**	**Surfactant**	**Phosphate**	**Silica**	**Soda ash**	**Surfactant**	**Phosphate**
***Soap solution**	2.94	0.97	0.03	ND	2.94	0.97	0.03	ND
Fresh oil	-	-	-	5.67	-	-	-	5.67
Incubation								
Fresh wastewater*	0.21	0.35	0.01	ND	0.21	0.35	0.01	ND
1	1.75	0.68	0.03	ND	2.12	0.29	3.65	14.1
2	1.54	0.71	ND	ND	1.25	0.45	2.58	2.45
3	2.20	1.05	0.02	ND	0.35	0.37	8.60	1.27
4	1.50	0.80	ND	ND	0.33	0.51	10.3	1.18
5	0.40	0.50	ND	ND	0.10	0.66	13.3	27.4
6	0.48	0.49	ND	ND	0.20	0.40	ND	9.76
7	1.10	0.47	0.01	ND	1.05	0.48	14.7	45.1
8	0.28	0.58	11.3	25.3	0.21	0.36	12.4	26.7
9	0.23	0.44	0.21	7.41	1.21	0.59	0.03	ND
10	0.20	0.40	10.0	24.8	0.49	0.39	ND	ND
11	1.24	0.43	ND	ND	1.16	0.40	25.4	152.9
12	1.21	0.54	ND	ND	2.35	0.45	13.5	27.8

Legend: ND = Not detected; *: Soap solution (mg of detergent[1]/ml of water) used for washing of dishes and food wastes [1]Omo: 15.6mg/100ml

Surfactant decreased in value but peaked up on the eighth day (11.3mg.l^{-1}). Phosphate was not detected initially but there were appreciable amounts (25.3 mg.kg^{-1} and 24.8 mg.kg^{-1}) on the eighth and tenth days respectively. This was due to the decomposition of organic residue of the food particles that were present in the wastewater culture. During anaerobiosis, the amount of soda ash decreased as the storage period increased. However, appreciable amounts of soda ash were detected on the fifth (0.66%), ninth (0.59%) and twelfth (0.45%) days of storage. The amount of surfactant was 8.60 mg.kg^{-1}, 13.3 mg.kg^{-1} and 25.4 mg.kg^{-1} on the third, fifth and eleventh days of storage. Meanwhile, silica increased gradually by the seventh (1.05%), ninth (1.21%) and twelfth (2.35%) days. Similarly, phosphate was appreciably extracted from wastewater samples on the fifth (27.4mg.kg^{-1}), seventh (45.1 mg.kg^{-1}) and eleventh (152.9 mg.kg^{-1}) day of incubation.

The composition of wastewater was always influenced by major changes in the redox and pH conditions that occur in the reaction zones of septic systems as reported earlier (**Sheryl et al., 1994**). In general phosphate retention is greater in acidic settings than in neutral or basic settings (**Sheryl et al., 1994**). Oxyhydroxides associated with Phosphates usually resulted into acidic environments whenever conditions change from aerobic to anaerobic. Phosphorus released under anaerobic conditions has been observed in surface water sediment (**Sheryl et al., 1994**).

The proximate components of the wastewater samples cultured aerobically and anaerobically over a period of 12 days were also determined. The proximate values for the fresh oil (without detergent, food particles and soup ingredients) are ash (0.36%), moisture (0.36%), fibre (ND), protein (1.90%), fat (85.6%), carbohydrate (11.7%) and oil (5.76g/100g). The proximate values for the aerobic culture ranged between 0.23-2.50% for ash, moisture (77.30-98.40%), fibre (3.79-11.8%), protein (1.53-5.51%), fat (0.20-2.30%), carbohydrate (1.00-1.03%) and oil (1.02-2.42%). The values for the anaerobic culture ranged between (0.12-2.35percent) for ash, moisture (80.80-97.40%), fibre (2.17-9.59%), protein (1.88-4.55%), fat (0.16-2.65%), carbohydrate (1.00-1.02%) and oil (1.06-2.42%) (Table7). As reported earlier, **Tchobanoglous et al. (1991)** indicated that the organic matter in wastewater is mostly composed of proteins and carbohydrates and smaller amounts of lipids and the rate of microbial activity (fermentation) on the fibre content of the wastewater sample may contribute to the slight increase in the carbohydrate and ash contents.

Tab 7 Proximate component of wastewater culture during storage

Incubation Period	Aerobic							Anaerobic						
	Ash (mg)	**Moisture (mg)**	**Fibre (%)**	**Protein (%)**	**Fat (%)**	**Oil (mg)**	**Carbohyd-rate (mg)**	**Ash (mg)**	**Moisture (mg)**	**Fibre (%)**	**Protein (%)**	**Fat (%)**	**Oil (mg)**	**Carbohyd-rate (mg)**
Fresh oil	0.36	0.36	ND	1.90	85.6	5.76	11.7	0.36	0.36	ND	1.90	85.6	5.76	11.7
Fresh wastewater	0.25	90.70	4.65	3.20	1.18	2.42	1.02	0.25	90.70	4.65	3.20	1.18	2.42	1.02
1	0.23	91.50	3.79	3.31	1.17	2.35	1.00	2.10	80.80	9.59	4.55	2.23	2.26	1.00
2	1.57	87.80	5.67	2.47	1.03	1.65	1.00	1.35	87.10	5.10	3.81	2.65	1.60	1.00
3	2.21	80.20	10.4	5.01	2.15	2.03	1.00	1.32	90.20	3.76	3.71	1.03	1.91	1.00
4	1.56	87.20	6.41	3.62	1.21	2.10	1.00	0.35	89.80	5.08	3.70	1.02	1.92	1.00
5	0.46	88.70	5.66	2.56	2.30	1.99	1.02	0.12	90.40	4.80	3.60	1.06	1.75	1.02
6	0.51	98.00	3.85	2.55	0.75	1.96	1.02	0.24	97.40	2.17	1.88	0.04	1.55	1.02
7	1.20	88.10	5.92	2.31	2.25	1.43	1.03	1.00	88.50	5.74	2.21	2.00	1.52	1.00
8	0.50	90.20	4.92	3.20	1.21	1.38	1.02	0.28	94.50	2.74	2.27	0.16	1.50	1.00
9	2.50	77.30	11.8	5.51	1.82	1.32	1.02	2.35	86.70	6.67	3.55	1.57	1.43	1.00
10	0.25	98.40	3.97	1.53	0.20	1.22	1.02	0.50	97.30	3.41	2.32	0.54	1.34	1.00
11	1.26	91.80	4.07	2.04	0.80	1.02	1.02	1.20	91.90	4.07	2.04	0.80	1.07	1.00
12	1.24	86.80	6.59	3.44	1.88	1.03	1.03	1.15	86.10	6.98	2.62	1.32	1.06	1.02

CONCLUSION

In conclusion, this study has shown that waste water needs proper and adequate treatment before dislodging into the environment. The degradation of saturated fatty acids was found to be limited in fat and oil-containing wastewater at a very high loading rate. This could be applied for practical fatty oil-containing wastewater generated from daily kitchen activities, such as from fast-food restaurants. In addition, the use of microorganisms characterized by such saturated fatty acid degradation may lead to treatment at a higher loading rate.

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