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BIO-TREATMENT OF LIPID-RICH WASTEWATER BY LIPOLYTIC BACTERIA FROM ABATTOIR ALONG ITS RECEIVING RIVER, (ABA RIVER), NIGERIA

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ABSTRACT

The heavy organic contents most especially lipids, in abattoir wastewaters discharged into receiving rivers, are higher than the WHO permissible level for discharge into water bodies. Hence the potency of lipolytic bacterial isolates and consortium in the effective biodegradation of lipid-rich wastewater from abattoirs was investigated. The lipolytic bacterial isolates were identified as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella pneumoniae*. The consortium used in the treatment was formulated comprising *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. The efficiency of the individual isolates and consortium treatment was measured by the chemical oxygen demand (COD), biological oxygen demand (BOD), lipid content and pH variation analyses. Within 18days of treatment under aerobic condition, the BOD values were reduced from 1075.40 mg/l to <20 mg/l for the consortium, *Staphylococcus aureus* and *Pseudomonas aeruginosa* treated wastewaters while >20 mg/l for *Bacillus subtilis*, *Klebsiella pneumoniae* and the control; the COD values were reduced from 1904.30 mg/l to >200 mg/l for *Staphylococcus aureus*, *Pseudomonas aeruginosa*,*Bacillus subtilis*, and *Klebsiella pneumoniae* while < 200mg/l for the consortium; lipid content reduced from 17006 mg/l to <20 mg/l for *Pseudomonas aeruginosa* and consortium, and >20 mg/l for others. The pH values of the treated media varied during treatment period experiencing a fall and rise. The lipolytic isolates have desirable features that could be favourably exploited for the treatment of lipid-rich wastewater from abattoir. This lipolytic consortium has proved to be beneficial for bringing down the overall organic load of this wastewater.

Keywords: Lipid-rich wastewater, Lipolyticbacteria, Consortium, Abattoir, Organic content

1.0 INTRODUCTION

1.1 Background of the Study

Wastewater is a by-product of residential, commercial, agricultural, or industrial waste production such as that from homes, farms, and manufacturing plants, among others. Lipids are general collection of fats, oils and grease, which are one of the major organic matters typically found in municipal, domestic, and some industrial wastewater generated from edible oil refineries, slaughterhouses, wool scouring, restaurants, bakeries, dairy product industries and food processing industries and make up a significant portion of problematic wastewater debris(Reuter *et al*., 2013). Lipid-rich wastewater, when released into the environment without adequate treatments, may provoke severe environmental problems or pollutions similar to that of petroleum oil due to common physical properties. Thus, lipids are substances of difficult biological treatment at cleaning systems and generate technological and environmental problems (Fadile*et al*., 2011). All these problems are partially due to lipids insolubility and

density contrasting the aqueous phase (Odeyemi *et al*., 2010). It is for this reason that there have been major efforts focused on the removal of lipids from wastewater early in the processing, prior to its movement downstream.Current industrial practices have led to an enormous generation of various crude lipid materials as waste,particularly lipid-waste from abattoirs.In Nigeria, many abattoirs dispose their effluents directly into streams and rivers without any form of treatment, and the slaughtered meat is washed by the same water (Adelegan, 2002). However, these lipid-containing wastewaters are generally contaminated with high levels of undesirable fatty acids.

Selection of microorganisms with a high degradation activity for lipids and their application for the removal of pollutants may be one of the ways to enhance biodegradation. Thus, isolation and identification of new effective strains with high lipase activity, a study of the mechanisms of lipase synthesis and secretion is particularly relevant for several reasons. First, lipases play a key role in the metabolism of strains that destroy lipids. Second, a poor performance of indigenous microorganism in lipids polluted environment could be effective by using bioaugmentation strategy (El Fantroussi*et al*., 2005) which needs additions of new effective strains. Third, some inoculated microorganisms in modern biotechnological preparations produced for biological treatment of lipid wastes, are not resistant in open systems and require periodic additions of inocula to achieve a high performance treatment (Loperena*et al*., 2007).

Previous works, have studied the application of microbial cultures with a high hydrolytic activity on hydrocarbons for cleaning the environment from petroleum contamination. Hence, there results lead to the presumption that such might be applicable in improving the biological treatment of the environment, particularly of wastewater rich in lipids. Therefore, this study is aimed at evaluating the potency of isolated lipolytic bacteria in the effective degradation of lipids from lipid-rich wastewater from Abattoir along the Aba River, Ogborhill, Aba.

2.0 MATERIALS AND METHODS

2.1 Study Area
Aba River is an important economic river in Nigeria. Aba town lies between latitude 50 03’N to 50 07’N and longitude 70 17’E to 70 24’E in Abia State of Southern Nigeria. The river serves as a major source of freshwater for the city. The river is used for various human and industrial activities and receives wastes from the industries especially the abattoirs sited along its course.

2.2 Collection of Wastewater Samples
Wastewater sample was collected aseptically from the major point of discharge to the Aba River into20litres sterile container.

2.3 Bacterial Screening, Identification and Characterization
Lipolytic activity of bacteria isolates were detected using tributyrin agar plates and clear zones around the colonies were observed.Following presumptive bacterial identification using Gram staining and cell morphology, biochemical analyses for the lipolytic bacterialidentification was carried out using the Analytical Profile Index (API) system(Biomerieux, France), API 20NE for the Gram negative bacterium and API 50CHB combined with API 20E for the Gram positive isolates according to the Global Health Laboratories (2013).

2.4 Physico-Chemical Properties of the Wastewater Samples
Prior to the wastewater treatment, the sampled wastewaters were subjected to some physico-chemical analyses. Samples were analysed for the following physicochemical parameters: pH, temperature, turbidity, total suspended solids, total dissolved solids, electrical conductivity,

protein concentration, reducing sugar concentration, lipid content, biochemical oxygen demand (BOD) and chemical oxygen demand (COD), according to methods used by Adesemoye (2006). The pH values of the samples were determined with a pH meter and temperature was determined with the mercury thermometer immediately after sample collection. Turbidity was determined by nephelometric method using a portable turbidity meter. Gravimetric method involving filtration and evaporation were used to measure lipid contents, total suspended solids and total dissolved solids. Electrical conductivity was determined using a conductivity meter. Measurement of BOD and COD, protein concentration and reducing sugar concentration was carried out as follows:

2.4.1 Determination of Chemical oxygen demand (COD)

Titrimetric method was employed in the determination of COD. A 10ml of 0.125M $K_2Cr_2O_7$ was added to 20 ml of the water sample using a pipette in a refluxing flask. Glass beads were added. Then 30 ml of concentrated H_2SO_4 was added slowly and with gentle swirling. The flask was connected to the condenser and refluxed for 2hours. After that, the flask was cooled and the condenser washed with distilled water into the flask and diluted to about 150ml. The excess dichromate was titrated with 0.05M ferrous ammonium sulphate (FAS) using 2 drops of ferroin as indicator. A blank mixture was prepared and treated using the same procedure (Ademoroti, 1996).

2.4.2 Determination of Biological Oxygen Demand (BOD)

BOD bottles containing 300 ml of properly diluted samples, which had been previously air-blown for 10 minutes, were incubated in the dark at 20^{o}C for 5 days prior to the determination of dissolved oxygen using the azide method (Helrich, 1990). This was done by adding 2ml of $MnSO_4$ and alkaline iodine–sodium azide solution to each of the BOD bottles. After placing the stoppers and expelling air-bubbles, the bottles were inverted several times and left for precipitation. Then, 2ml of H_2SO_4 was added and mixed by inverting the bottles until iodine is uniformly distributed. Starch indicator will be added to a sample of 2ml and, then, the solution was titrated with 0.025M $Na_2S_2O_3$ until the blue colour disappeared. The volume of $Na_2S_2O_3$ added was used in calculation for the BOD value.

2.4.3 Determination of Lipid content

Lipid content was determined by the partition-gravimetric method (Kirschman and Pomeroy, 1949). A 250-ml sample acidified to pH 2.0 with1:1 diluted HCl was used for each assay. Lipid was extracted repeatedly with 30 ml portions of 1, 1, 2-trichloro-trifluoroethane (Freon) until the aqueous phase showed no oil layer and the solvent phase was clear. The combined solvent extracts were evaporated at 70^{o}C and, then oven-dried overnight at 70^{o}C. The dry weight obtained indicated the amount of oil and grease present in the sample.

2.5 Wastewater Treatment

Wastewater treatment was done according to Mongkolthanaruk and Dharmsthiti, (2002) with modifications. Wastewater sample was distributed into six portions of 2 litres each contained in 2 litres flasks. In five containers, 20ml was inoculated of each bacterial culture (OD at 600nm of 3Mcfarland standard equivalent approximately cell density 6×10^{8}cfu/ml) to make 1% (v/v) while one container was left uninoculated, thus control. The flasks were kept in shaking incubator at 35˚C with 200 rpm. Samples were drawn from each of the flasks at regular intervals of 72hours for BOD, COD, lipid content and pH analyses.

3 RESULTS AND DISCUSSION

3.1 Physicochemical Characterisation of the Wastewater Sample

Parameters	Results	WHOstandard
Colour	Pale red	
Appearance	Clouded	
pH	5.92±0.14	6.00
Temperature (^{o}C)	29.02±0.04	
Turbidity (NTU)	418.55 ±1.18	
Conductivity (µs/cm)	394±6.24	
Total Soluble Solute (mg/l)	1560.8±0.67	20
Total Dissolved Solute (mg/l)	981.1±0.26	200
Biological Oxygen Demand (mg/l)	1075.4±1.86	20
Chemical Oxygen Demand (mg/l)	1904.3±0.61	200
Lipid Contents (mg/l)	17006±2.08	20
Protein Determination (mg/l)	744.37±1.48	
Reducing Sugar (mg/l)	461.89±0.03	

3.2 Bacterial Identification and Lipolytic Screening

The lipolytic screening on tributyrin agar plates, four of the isolates was seen with zones of clearance around their colonies confirming their lipolytic ability.These isolates were identified as *Pseudomonas aeruginosa*, *Staphylococcus aureus, Bacillus subtilis, and Klebsiellapneumoniae.*

3.3 Wastewater Treatment

After treating the wastewater with individual bacteria and the consortium of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*, the values of BOD of the wastewater sample reduced based on the degrading capacity of the organisms (Figure 1). On the period of 15th day of incubation, all the treated wastewater showed great reduction of the BOD to 28, 35, 65, 95 and 19 mg/l for *Pseudomonas aeruginosa*, *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae* and consortium respectively.

After treatment of the wastewater with the individual bacteria and consortium, progressive reduction of COD in all the treatments was obtained (Figure 2), according to the utilization of the organic matter by the organisms.

The content of lipids in all the treated wastewater samples decreased at the end of the 18thday of treatment (Figure 3) according to the degrading abilities of the organisms.

3.1 pH variation at wastewater treatment

During the wastewater treatment, pH varied with incubation periods based on the degrading capacity of the bacterial treatment. All the treated wastewaters were seen to exhibit similar trend of pH variation from being more acidic to less acidic or alkaline (Figure 4).

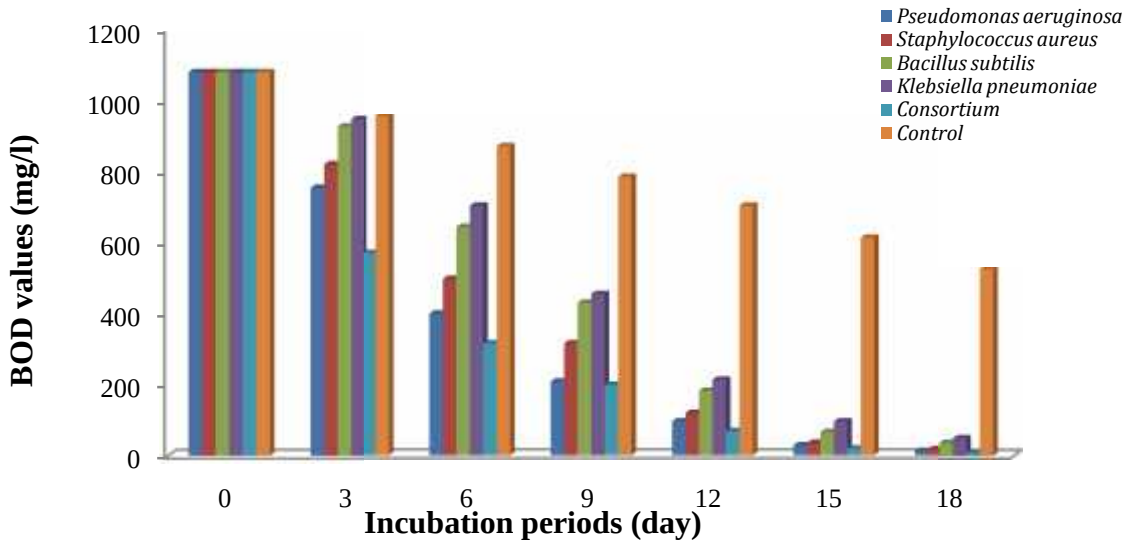

Figure 1: BOD during wastewater treatment at different incubation periods

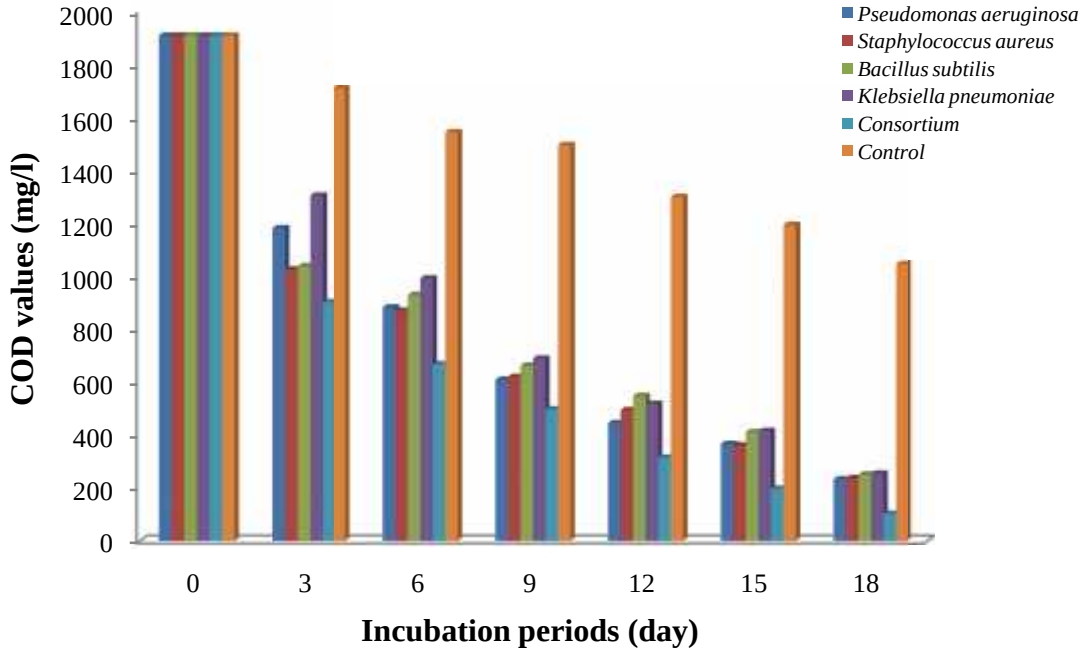

Figure 2: COD during wastewater treatment at different incubation periods

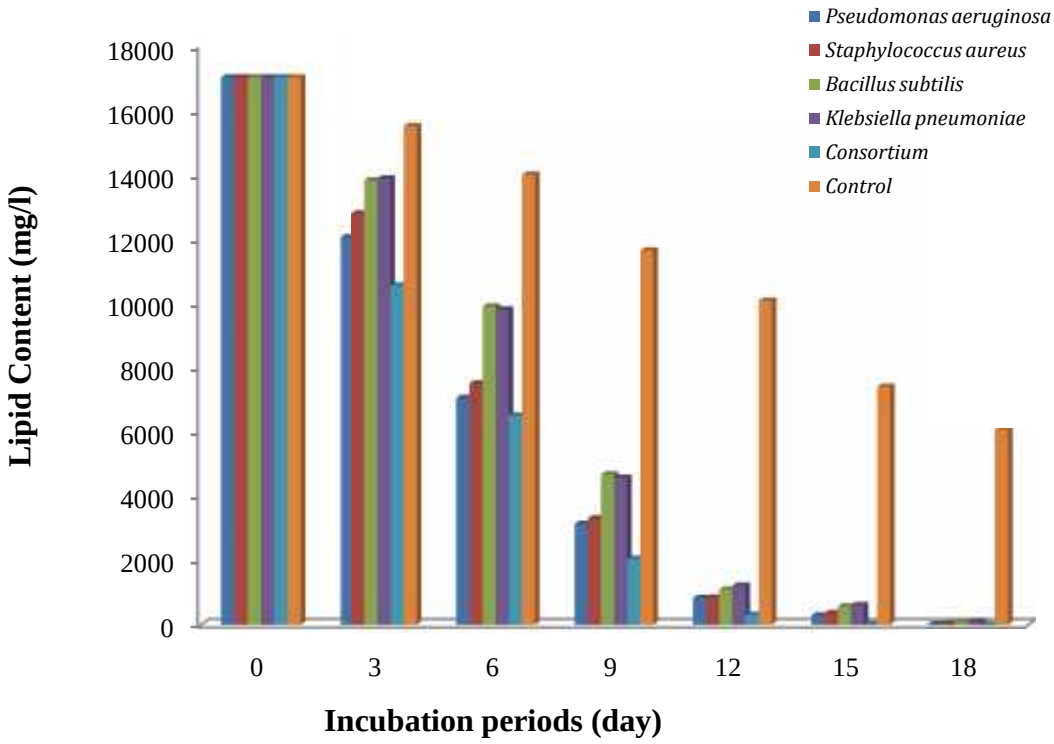

Figure 3: Lipid Content during wastewater treatment at different incubation periods.

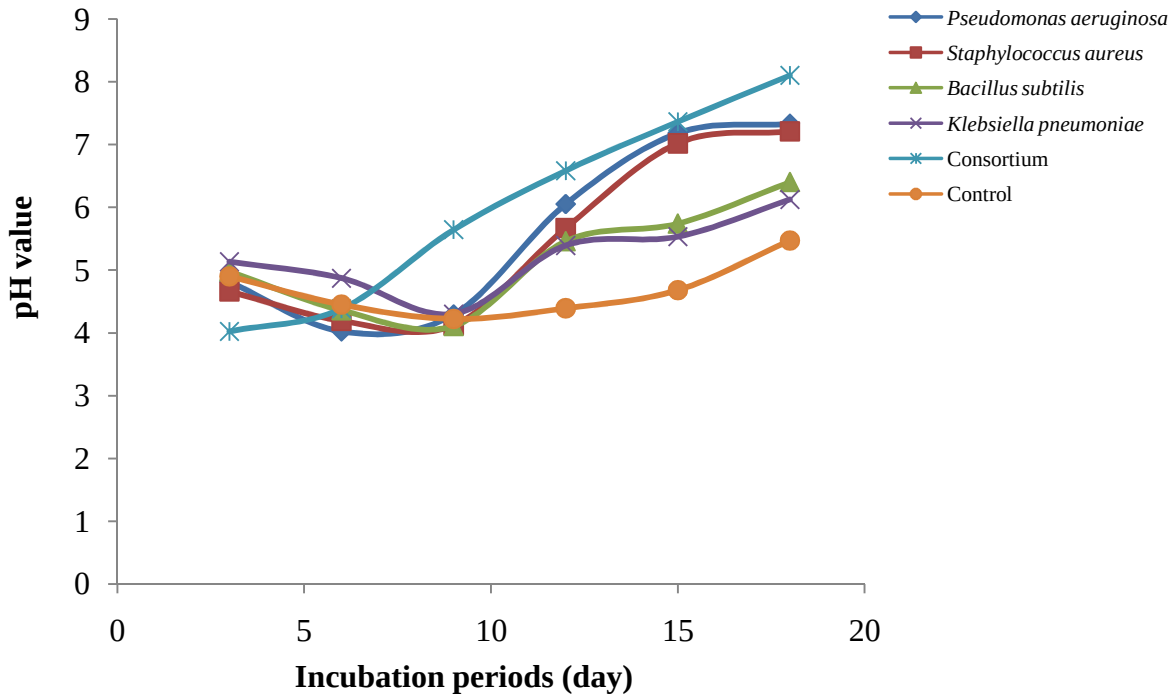

Figure 4: pH variation during the wastewater treatment at different incubation periods

4 DISCUSSION

4.1 Physicochemical characteristics during treatments

The mean values of the BOD, COD, and Lipid content (Table 4.1) exceeded the WHO/USEPA permissible limits, for the discharge of the wastewater from abattoir industries into rivers. Although, many researchers has reported several values of properties of different abattoirs wastewaters (Akan *et al.,* 2010; Masse and Masse, 2000; Adesemoye *et al.*, 2006), some of the results obtained in this study was similar to that reported by Ogbonna and Ideriah, (2014),which showed results of range of 6.71-9.37 for pH, 20-30.4^{o}C for temperature, 165-6080 mg/l for Total soluble solids (TSS), 155-1560 mg/l for Total dissolved solids (TDS), 75-1200 mg/l for biochemical oxygen demand (BOD), 150.2- 9265 mg/l for Chemical oxygen demand (COD); and 25000 mg/l for the Lipid content (Prasad and Manjunath, 2011).

The high concentration of lipids in the wastewater samples was from the lipid content of the part of animals slaughtered in the abattoir. From every indication, the observed high Chemical Oxygen Demand in this study can be attributed to lipids and other constituents of the abattoir processes such as blood and faeces that were washed into the source water. Thus, the organic pollutants will create high competition for oxygen within the ecosystem. COD is a determinant used to assess organic pollution in aqueous ecosystem and is one of the most important parameters in water monitoring (APHA, 1998). Similarly, the high BOD value observed shows that high oxygen is required for biological degradation of organic materials. It depicts the amount of putrescible organic matter degraded by microbial metabolism on the assumption that the water has no bactericidal or bacteristatic effects (ALPHA, 1998).

The acidic pH observed may be attributed to some chemical reactions associated with dissolution, formation or alteration of minerals, such as chemical reaction between blood and water, or organic/inorganic matter from animal faeces with water. Thus pH is important to microorganism because it affects the functioning of virtually all enzymes, hormones, and proteins which control metabolism, growth and development (Ogbonna and Ideriah, 2014).

4.2 Wastewater Treatments

In this study, *Pseudomonas aeruginosa*treated wastewater sample showed very good reduction of BOD, COD and Lipid content values from the first day. This result is similar to the report of Dharmsthiti and Kuhasuntisook (1998), where *Pseudomonas aeruginosa* LP_{602} strain a lipase producing bacteria proved to have high potential for use in lipid-rich wastewater treatment for kitchen wastewater samples, also *Pseudomonas aeruginosa* was observed to be efficient in palm oil effluents, soap effluents, dairy and domestic effluents (Prasad and Mangunath, 2011). Also, *Staphylococcus aureus* treated wastewater, resulted in lowering the BOD, COD, lipid content as well, which was similarly reported by Prasad and Mangunath, (2011) to show lower BOD and lipid content in slaughterhouse wastewater than other organisms used in the treatment. As observed, the *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated produced a high efficient lipolytic activity which correlates with those of Prasad and Mangunath, (2011).

4.3 Consortium Treatment

The use of combined culture of *Pseudomonas aeruginosa* LP_{602} and *Bacillus subtilis* B_{304} could not reduce BOD and the Lipid content in the wastewater to an acceptable level for environmental discharge, except the addition of a third strain of lipase producer bacteria *Acinetobactercalcoaceticus*LP_{009} (Pratuangdejkul and Dharmsthiti, 2000), facilitated the bioremediation process. In this study, a combined culture of the three organisms that make up the consortium (*Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis)* showed maximum activity with very low BOD, COD and Lipid content values compared with

individual bacterial treatment of the wastewater. The resultant values of these parameters in this treatment were acceptable for wastewater discharge into the environment, which shows that the consortium of the three bacterial cultures could be successfully used for lipid rich wastewater treatment.

4.4 Indigenous Treatment

It was found that waste degradation by natural, indigenous microbes already present in the wastewater did occur at very low efficiency as the BOD, COD and Lipid content still remained high and will not be acceptable for discharge into the environment (Figures 1, 2, and 3). This could be as a result of low lipolytic activity. Other indigenous microbes that are not lipolytic may suppress the lipolytic microbes, or limit the nutritional substrate available, or the produce toxic substances of metabolism, for example, long chain fatty acid, that may antagonize the growth of the organisms to enhance degradation. As reported by Matsumiya*et al.,* (2007) that lipolytic microorganisms sometimes lose their ability to degrade fats in open treatment system due to the effects of indigenous microbes.From this result, it is suggested that use of an effective intensified bioaugumentation strategy which needs addition of new effective strains to overcome the poor performance of indigenous microorganisms in lipid polluted environment (El Fantroussi*et al.,* 2005).

4.5 Variation in pH during wastewater treatment

The result of variation of the pH is similar to that observed by Fadile*et al.,* (2013). The pH of the wastewater reduced from the initial pH value of slightly acidic to a more acidic pH value from the 1^{st} day to the 6^{th} day (Figure 4). This reduction in pH observed in the result could be due to the metabolism/degradation of lipids which prompts the release of the fatty acids and glycerol, and the ions H^+ by the cells in the medium through the Kreb's cycle. The cellular activity (glycolysis and Kreb's cycle) thus appears by a fall of the pH, whereas its increase could indicate that the cells are under physiological stress (Fadile*et al.,* 2013). Then, the pH increases gradually during the following days then, greatly by the 12^{th} day until the 18^{th} day when it reaches slightly neutral in treated wastewater with *Bacillus subtilis* and *Klebsiella pneumoniae* but greater than neutral in the other treatments (Figure 4). This could be due to hydrolysis of the fatty acids and organic acids having groupings COO^- and OH^-. The disappearance of these compounds involves a rise in the pH of the medium, although, the microbial activity may involve a degradation of proteins by releasing the ions NH_4^+ which causes the increase in the pH (Fadile*et al.,* 2013).

5.0 CONCLUSION

The results obtained revealed that, the lipolytic isolates have desirable features that could be favourably exploited for the treatment of lipid-rich wastewater from abattoir processes also the importance of the bacterial consortium in processing the wastewater as it proved more effective in treatment.

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