Biotreatment of Slaughterhouse Waste Water by Microalgae

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***Abstract*—** Slaughterhouse wastewaters are characterized by a high organic content, mainly composed of proteins and fats. Therefore, these wastewaters should be treated efficiently prior to discharge into receiving bodies to avoid severe environmental pollution. The use of natural remediation methods to remove contaminants from waste water is becoming more popular. One of the aims of waste water treatment is to reduce nutrient such as nitrate and phosphate level in effluent to a protective level of the receiving water body. This work aimed to characterize poultry slaughterhouse wastewater generated in Mobi area, in Ede South local government and assessing wastewater treatment plants performance by Chlorella vulgaris ChA and feasibility of wastewater reuse. Freshly discharge poultry slaughterhouse waste water sample (PSHWWS) was collected from Mobi area in Ede and analyzed for the physicochemical parameters such as pH, Total Dissolved Solid (TDS), Temperature, dissolved oxygen, total Alkalinity, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), ammonia, phosphate and Nitrate by standard methods. 190ml of the sterilized PSHWWS was inoculated with 10ml of C. vulgaris. The inoculated sample was incubated under 2ft cold fluorescence light for two weeks and determines the physicochemical parameters at 7 days interval. The results observed for raw, bio treated and removal efficiency showed: pH (7.04 and 8.45), TDS (521.45 and 121.80mg/l with 76.64%), Temperature (30.27 and 27.47oC), Dissolved oxygen(4.85 and 0.95 mg/l with 80.41%), Total Alkalinity (143.08 and 55.84 mg/l with 60.97%), BOD(1480.05 and 486.81mg/l with 67.11%), COD(1778.24 and 56.84mg/l with 96.80%), ammonia (78.30 and 33.09 mg/l with 57.74%, phosphate (16.54 and 7.83 with 56.66% and nitrate (454 and 9.29 mg/l with 97.95%). Chlorella vulgaris showed a potential removal of pollutant and other wastes from the slaughterhouse waste water.

***Keywords* —** Poultry, slaughterhouse, waste water, bio treatment, Chlorella. vulgaris ChA

I.INTRODUCTION

Discharge of waste water from poultry slaughterhouse is one of the sources of environmental pollution. The composition of poultry wastes water is not environmental friendly rather they are hazardous to immediate environment and aquatic environment. The adverse effects of this wastes water are not to animals and living aquatic alone but have affected human life through consumption of water contaminated with this wastes as well as spread of diseases. Most practices in our slaughterhouses do not give room for treatment of wastes before they are released to the environment. Few slaughterhouses that prioritize the treatment of poultry slaughterhouses before discharge to immediate environment are either involves in partial treatment or indirectly aggravating the resultant problems links to waste water as a result of chemical used in treatment (Akpors and Muchie, 2011).

Poultry slaughterhouse wastewater (PSW) pre-treatment is germane before sequential biological treatment and the release of such treated wastewater into freshwater bodies. If not pre-treated, the PSW may contribute to the pollution of the environment, and therefore the contamination of fresh water sources, which can culminate in a negative impact on human health and aquatic life. The PSW contains a high concentration of lipids (fats, grease, oil and fatty acids) as a major component of organic matter (Commarota and Freire, 2006). Therefore, the implementation of pre-treatment process (es) is necessary as it is an important step in improving the wastewater quality prior to further treatment using anaerobic biological systems. Suitable pre-treatment systems have been developed for lipid-containing wastewater from slaughterhouses, with such techniques being applied prior to the use of primary and secondary treatment processes, such as anaerobic digesters for organic matter reduction, nitrification, and subsequent denitrification for total nitrogen reduction (Abdel-Rand *et al*., 2012).

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Pre-treatment processes such as dissolved air flotation systems (DAFs) and grease traps are often applied (Tanikawa *et al*., 2016). However, complications can occur during their utilisation, contributing to the inefficient removal of lipids which will buildup in the sludge used in anaerobic processes, reducing their effectiveness to treat the PSW (Commarota and Freire, 2006). Overall, influent of improperly pre-treated high-lipid content PSW to downstream wastewater treatment processes may impede both aerobic and anaerobic downstream processes (Rigo *et al*., 2008). In an aerobic processes, a layer of lipids may be formed which will interrupt pollutant transformation by the bioremediating biomass,and also decrease access to dissolved oxygen (DO) in aerobic processes such as nitrification (Bustillo-Lecompte *et al*., 2016).

Furthermore, operational damage which culminates in process redundancies, due to solidified lipids at low temperatures during anaerobic treatment, has been reported in numerous studies for slaughterhouse wastewater (Commarota and Freire, 2006; Valladão et *al.*, *2007*). Even after successful primary pre-treatment, further lipid removal might be required in a process which is environmentally benign. When a DAF system is utilised as a pre-treatment system, 60-85% of lipids can be removed (Massé and Masse, 2000), with the rest passing down to downstream processes. Clearly, the remaining lipids will thus accumulate in these downstream PSW bioremediation systems, which will effectively reduce the efficiency of such processes overtime. The use of alternative biological methods with current pre-treatment systems involving enzymes has been referred to as a promising alternative for further FOG reduction in effluent from pre-treatment processes, a technique suitable for high lipid-containing wastewater such as PSW (Rigo *et al*., 2008).

Biotreatment involves uses of naturally occurring biological means and microorganisms to treat wastewater of its nutrients. Biological wastewater treatment is mainly carried out by Prokaryotes, Plants (*Lemna* sp., water hyacinth, vetiver grass, hydrilla grass), Microalgae, Protozoan and Rotifers (Bitton, 2005). Biological wastewater treatment is therefore of utmost importance for the wellbeing of water bodies. This calls for a continuous development and refinement of wastewater treatment techniques as part of the effort to make the world a cleaner place. Microalgae are microscopic photosynthetic organisms that are found in both marine and freshwater environments. Their photosynthetic mechanism is similar to land based plants, due to a simple cellular structure, submerged in an aqueous environment where they have efficient access to water, carbon dioxide and other nutrients, they are generally more efficient in converting solar energy into biomass. The use of a wide range of microalgae such as *Chlorella*, *Scenedesmus*, *Phormidium*, *Botryococcus*, *Chlamydomonas* and *Spirulina* for treating wastewater has been reported and efficacy of this method is promising (Stephens *et al*., 2010).

The advantage is that while the microalgae will be removing excess nutrients in the wastewater, there will be concomitant accumulation of biomass for downstream processing (Chinnasamy *et al*., 2010). In a study by Zhang *et al*. (2008) *Scenedesmus* sp. showed high removal efficiency for inorganic nutrients such as nitrates and phosphate from domestic effluents. The potential for microalgae in waste water remediation is however much wider in scope than its current role (De-Bashan and Bashan, 2010). Algae, particularly green unicellular microalgae have been proposed for a long time as a potential renewable fuel source (Oswald and Golueke, 1960). In addition, waste water treatment by microalgae is an eco-friendly process with no secondary pollution as long as the biomass produced is re used and allows efficient nutrient recycling (Godos *et al*., 2003).

Physical and chemical pre-treatment systems are employed for the delipidation of protein- rich wastewater from poultry slaughterhouses prior to the biological treatment of such wastewater for the overall reduction of FOG, and therefore of total chemical oxygen demand (COD). However, these treatment techniques are capital intensive and contribute to the accumulation of toxicants in wastewater treatment processes as chemical compounds, e.g. synthetic chemical flocculants, are used for the removal of FOG. Hence, bio-treatment (Biological techniques) offer an innovative, cost-effective and environmentally benign alternative for the reduction of lipids contained in wastewater, such as that of poultry slaughterhouses. Bio-treatment of PSW using biological methods is a promising alternative pre- treatment technique, although it has not been studied extensively. The current study is focus on bio-treatment of poultry slaughterhouse waste water using *Chlorella vulgaris* ChA. The objectives of this research work includes: Analysis of the physicochemical parameters of poultry slaughterhouse waste water, Treatment of the poultry waste water with microalgae (*Chlorella vulgaris* ChA) and analysis of the physicochemical parameters of treated poultry slaughterhouse waste water

II.MATERIALS AND METHODS

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A. Poultry Slaughterhouse Waste water Sample

Waste water samples from Poultry slaughtered house waste water in Mobi Area, Ede South Local Government, Osun-state, were aseptically collected in laboratory clean and sterile containers. The area lies within Latitude 7.700ºN and Longitude 4.450ºE of the equator. The sample collected was then corked and transferred to the laboratory for analysis after 1-2hrs of sample collection.

B. *Microalgae sample*

The source of microalgae (*Chlorella vulgaris* ChA) for bio-treatment was water sample collected from University of Ibadan fish farm pond.

C. *Sterilization of Apparatus*

All apparatus used in this study were thoroughly washed with detergent, rinsed with water, air-dried and sterilized in hot air oven at 160ºC for two hours. All media used for isolation, cultivation and identification of micro algal isolates were sterilized by autoclaving at 121ºC for 15 minutes under pressure.

D. *Morphological characterization of microalgae*

Isolate was characterized morphologically with the aids of compound light microscope based on characteristics like shape, size, cellular structure i.e unicellular/multicellular, chloroplast, pyrenoid, motility and nucleated.

E. *Molecular Identification of Microalgae*

i. DNA EXTRACTION: The genomic DNA of Algae was isolated using the InstaGeneTM Matrix Genomic DNA Isolation kit. Below procedure was followed as required by the kit instruction. The colonies of Isolated microalgae were picked and immersed in a microfuge tube contained 1ml of sterile water. The supernatant was removed by centrifugation iat 10,000–12,000 rpm for 1 minute. This was preceded by addition of 200 µl of Insta Gene matrix to the pellet and incubation for 15 minutes at 56 °C. The tube was place in boiling water bath at 100 °C for 8 minutes after vortexing at high speed for 10 seconds. Finally, the content was vortex and spin at high speed for 10 seconds and 10,000–12,000 rpm for 2 minutes respectively. In result, 20µl of the supernatant was used per 50 µl PCR reaction.

ii. POLYMERASE CHAIN REACTION: Universal primers gene fragment was amplified using MJ Research .PTC-225 Peltier Thermal Cycler through 18S rRNA ITS Region

iii. PRIMER DETAILS: To 20µl of PCR reaction solution, 1µl of template DNA was added. Using 18S-C^a/18S-D^a/18S C-2^b/18S D-2^b primers The PCR reaction was performed using 18S-C^a/18S-D^a/18S C-2^b/18S D-2^b primers (Table 1) with below conditions : Initial Denaturation at 94ºC"for"2"min"and"then 35 amplification cycles at 90 ºC for 45sec, 50 ºC for 60sec, and 72 ºC for 60sec. Final Extension at 72 ºC "for"10"min. DNA fragments are amplified. Include a positive control (E.coli genomic DNA) and a negative control in the PCR

Table 1:18S rRNA PRIMERS USED FOR PCR REACTION

PRIMER	SEQUENCE
18S rRNA	
18S-C^a	5'-TGATCCTTCYGCAGGTTCAC-3'
18S-D^a	5'-ACCTGGTTGATCCTGCCAG-3'
18S C-2^b	5'-ATTGGAGGGCAAGTCTGGT-3'
18S D-2^b	5'-ACTAAGAACGGCCATGCAC-3'

iv. PURIFICATION OF PCR PRODUCTS: Montage PCR Clean up kit (Millipore) was used to remove unincorporated/unattached PCR primers and dNTPs from PCR products. The sequence of PCR product was done using 18S-C^a/18S-D^a/18S C-2^b/18S D-2^b primers. ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems) were used to performed sequencing reactions.

v. SEQUENCING PROTOCOL: On each template, Single-pass sequencing was performed using 18s rRNA universal primers. Ethanol precipitation protocol was used for purification of the fluorescent-labelled fragments from unincorporated terminators. The samples were subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems) after suspension in distilled water.

vi. BIOINFORMATICS PROTOCOL: The NCBI blast similarity search tool was used to blast the rRNA sequence . The phylogeny analysis of our sequence with the closely related sequence of blast results was

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performed followed by multiple sequence alignment. The programs MUSCLE 3.7 was used for multiple alignments of sequences (Edgar, 2004). The program G blocks 0.91b was used for curing of aligned sequences. The poorly aligned positions and divergent regions (removes alignment noise) were eliminated by G blocks (Talavera and Castresana, 2007). Finally, the program PhyML 3.0 a LRT was used for phylogeny analysis and HKY85 as Substitution model. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data, while being one order of magnitude faster. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data, while being one order of magnitude faster. The program Tree Dyn 198.3 was used for tree rendering. (Dereeper *et al*., 2008).

F. *Determination of Physicochemical Characteristics of Waste Water Samples*

The physico-chemical parameters of wastewater from Poultry slaughtered house in Mobi Area, Ede South Local Government, Osun-state was analyzed using standard analytical procedure (APHA, 1998). The physico-chemical parameters analyzed include; pH, total dissolved solid, temperature, dissolved oxygen (DO), total alkalinity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia, phosphates and nitrate were also analyzed. The procedures involved in carrying out the physico-chemical processes are discussed below:

i. DETERMINATION OF pH: The pH of the waste water sample was determined using a pH meter (Toledo, MP220). Each water sample was measured into 100 cm^3 beaker and the pH determined by inserting the pH meter probe after standardization into the beaker and taking the reading. Standardization of the meter was ensured after each reading (AOAC, 2006).

ii. DETERMINATION OF TOTAL DISSOLVED SOLIDS: Total Dissolved Solids (TDS) for each water sample was determined mathematically as a product of conductivity multiplied by a constant value, 0.6 (APHA, 1985).

TDS = Conductivity × 0.6.

iii. DETERMINATION OF TEMPERATURE: The temperature of waste water sample was determined using a simple Mercury-In-Glass thermometer (Assistant, DIN 12770) calibrated in degrees centigrade as described by Edema *et al* ., 2001 and Dinrifo *et al*., 2010.

iv. DETERMINATION OF DISSOLVED OXYGEN (DO): Dissolved Oxygen (DO) was determined using the Dissolved Oxygen meter (Model OXi315i), WTW82362. The dissolve oxygen meter was dipped into a sample, allowed to be steady and the result was recorded (APHA, 1985).

v. DETERMINATION OF TOTAL ALKALINITY: Alkalinity determination was done by measuring fifty ml of each sample into a onical flask and two drops of sodium trioxosulphate (ii) added to remove traces of chlorine. Three drops of mrthyl orange indicator was then added and titrated with 0.02N tetraoxosulphate (vi) acid acid in the burette.

Titer value × 20 = Alkalinity (mg/l) (Anon, 2002).

vi. BIOCHEMICAL OXYGEN DEMAND: To determine the biological oxygen demand (BOD), two 100 ml bottles were obtained with lids and cleaned well. 25 ml sample was taken in each bottle and 75 ml of distilled was added to the two bottles and were tightly closed. One bottle was kept in the incubator at 20-22°C for 5 days. The 10 ml of Manganese sulphate solution and 2 ml of alkali- iodide solution were added to the other bottle below the surface of the liquid by using a syringe. Thereafter the bottles were closed and mixed by inverting the bottle several times. When the precipitate settles it leaves a clear supernatant above, the precipitate was shaken again slowly by inverting the bottle, and when the setting has produced at least 50 ml supernatant 8 ml of conc. H_2SO_4 was added. The bottle was closed and mixed by gentle inversion until dissolution was completed. 100 ml of the sample was titrated with 0.05M $Na_2S_2O_3$ solution until a pale yellow solution is reached, 2 ml of freshly prepared starch solution was added and titration continued until a blue colour appeared. The procedure was repeated using 100 ml distilled water (blank) and this was repeated for incubated sample after 5 days. The BOD was calculated as follows:

BOD as mg/L = 16($V1$ - $V2$)

Where:

$V1$ = ml of $Na_2S_2O_3$ used for the sample before incubation;

$V2$ = ml of $Na_2S_2O_3$ used for the sample after incubation.

vii. CHEMICAL OXYGEN DEMAND (COD): COD analysis was performed using pre-packaged mercury-

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free and premixed COD vials based on Section 5220 of *Standard Methods* (APHA, 1998, 2012). Three types of COD vials with the ranges 5- 150, 20-900 and 100-4,500 mgCOD/L were used accordingly. A COD reactor was preheated to 150°C before testing. During every test, a 2.5 mL sample was carefully added into one COD vial of ranges 5-150 or 20- 900 mgCOD/L, and 0.5 mL sample were carefully added into one COD vial of range 100 4,500 mgCOD/L. Then, the vial was thoroughly shaken by hand. COD standards and a DW blank were processed exactly the same as the samples. COD vials containing sample, COD standard, and blank, were heated in the COD reactor for 2 h at 150±2°C, and then they were removed from the reactor and placed in a rack until they cooled and any suspended precipitate in the vials settled down. After the outsides of vials were wiped to remove dust, the vials were placed into the Orbeco Hellige MC500 Multi-Parameter Colorimeter one by one, to measure their COD concentrations under a standard curve covering the expected range of sample concentrations. The wavelength of 440, 600, and 600 nm were set for the ranges 5-150, 20-900 and 100-4,500 mgCOD/L, respectively. According to the requirements of the test method for using the COD vials, blanks of the ranges 20-900 and 100-4,500 mgCOD/L were used to set the zero in the colorimeter before sample testing.

viii. AMMONIA NITROGEN (NESSLER METHOD): The Mineral Stabilizer complexes hardness in the sample, the Polyvinyl Alcohol Dispersing Agent aids the color formation in the reaction of Nessler Reagent with ammonium ions. A yellow color is formed proportional to the ammonia concentration. Test results were measured at 425 nm. A blank prepared from deionized water treated and measured equally as the sample (APHA, 1998).

ix. DETERMINATION OF PHOSPHATE: To 25ml of the sample was added to 0.5ml of ammonium molybdate and 2 drops of stannous chloride and mixed by swirling. A blue color developed within an hour and the intensity was measured using a spectrophotometer (21D) at 690 nm (APHA, 1998).

The concentration of the phosphate was calculated

Phosphate (mg/l) = A - B X C

Where; A = Absorbance of sample;

B = Absorbance of blank sample,

C = Volume of standard phosphate

x. DETERMINATION OF NITRATE: Test tube was filled with sample to 20ml mark and one level spoonful (~ 1.5 ml) of nitratest powder (containing zinc dust 60% and barium sulphate 40%) and one nitratest tablet (ammonium chloride) were added and was shaken for a minute. The tube was allowed to stand for a minute and was inverted 3-4 times to aid flocculation and was allowed to stand for two minutes to ensure complete settlement. The clear solution was dispersed into 10 ml mark and one nitricol tablet (Sulfanilic acid, acting as the aromatic amine), was added, crushed, and mixed to dissolve, then it was allowed to stand for 10 minutes for color development and readings were taken on the Photometer (Wagtech) at 570 nm wave length.

G. *Experimental Set Ups for conventional bio treatment of wastewater*

To study the role of Microalgae (*Chlorella vulgaris* ChA*)* in wastewater treatment, the following methods were employed:

Poultry slaughtered house waste water + *Chlorella vulgaris* ChA

The experiment were conducted in triplicates and incubated under the same condition in 250 mL Erlenmeyer flask for period of 14 days.

H. *Inoculation and Sampling*

10 mL of exponential growing of *Scenedesmus obliquus* SeA was inoculated into three 250 mL Erlenmeyer flask containing 190ml of Sterilized Poultry slaughtered house waste water samples. Samples were taken for physicochemical analysis at interval of 7 days after inoculation for two weeks.

I. *Statistical Analysis*

Data obtained were subjected to appropriate statistical analysis.

III. RESULT AND DISCUSSION

A. *Morphological and molecular identification of microalgae*

Isolate UIA consists of small, non-motile unicells (rarely aggregated into small groups). The cells are spherical with a single, parietal, cup-shaped (sometimes plate-like) chloroplast with a single pyrenoid. The cell wall is generally thin, smooth and planktonic (Table 2). Isolate UIA was identified as *Chlorella vulgaris* after

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comparing its 18S rRNA sequence to similar sequences stored in the gene bank of NCBI. *Chlorella vulgaris* UIA is 100% closely related to *Chlorella vulgaris* strain SAG 30.80 (Figure 3).

Table 2: Morphological Characteristics of Microalgae

Morphological characters	Isolate
Shape	Spherical
Size	small
Unicellular/Multicellular	Unicellular
Planktonic/Benthic	Planktonic
Chloroplast	Parietal
Pyrenoid	Single
Motility	Non-motile
Nucleated	Uninucleated

Table 3: Molecular Identification of Microalage based on partial 18SrRNA gene sequence analysis

Isolate Code	Identity	Identity from BLAST	Percentage similarity (%)
SeA	*Chlorella sp*	*Chlorella vulgaris* SAG 30.80	100

B. *Physicochemical properties of Raw and treated poultry slaughtered waste water*

The mean pH values increased gradually from 7.04 to 8.45 for Mobi poultry slaughterhouse waste water (Figure 1). The pH of the raw poultry slaughterhouse waste water is 7.04, which is neutral, after 7 days of bio-treatment the pH increased to 7.85 and 8.45 after 14 days of treatment with *Chlorella vulgaris* ChA. The pH values obtained in this study were within the range of optimum pH levels for anaerobic digestion (Speece, 1996) and were within the World Health Organisation (WHO) tolerance limits of 6.0 to 9.0 for the discharged of wastewater into aquatic environment (Akan *et al*., 2010). The initial neutral pH (7.04) that characterized the onset of this work contradicted the observation made by Adesemoye *et al*. (2006) which recorded an acidic pH in characterization of sampled abattoir effluent. The anaerobic degradation of organic compounds releases ammonia, which react with carbon dioxide produced during the anaerobic process, resulting in ammonium bicarbonate, which contributes to the increase in pH values. This phenomenon according to Padilla-Gasca *et al*. (2011), can be attributed to a high concentration of organic compounds present in the poultry slaughterhouse wastewater which is composed mainly of proteins (like blood).

Total Dissolved Solid (TDS) recorded for Mobi poultry slaughterhouse waste water was 521.45 mg/L (Figure 2a). TDS values obtained were generally within 1000 mg/l the upper limit set by WHO (WHO, 2011). The value later reduced to 442.29 and 121.80 mg/L with removal efficiencies of 15.18 and 76.64% respectively after 7 and 14 days of treatment with *Chlorella vulgaris* ChA (Figure 2b). The electrical conductivity and total dissolved solid exhibited similar trend in both abattoir effluents, this is as a result of the linear relationship that exist between the two parameters (Radojevic, 1999). Chemical Oxygen Demand (COD) is considered as the amount of oxygen consumed by the chemical breakdown of organic and inorganic matter. The temperature of Mobi poultry slaughterhouse waste water is 30.27°C (Figure 4.3). There was a slight decrease in temperature (28.62 and 29.85°C) after 7 and 14 days of bio-treatment (Figure 3). The values observed after 7 and 14 days are slightly below the limit value of direct discharge into the receiving environment (30°C). Similarly, these values are considered indicative limit value for water for irrigation (35°C).

The dissolved oxygen (DO) in the various abattoir effluents were below undetectable concentrations before the end of the first 14 days interval. This observed change is due to the nature of the experimental setup and also as a result of increase in the microorganisms' activities which used up the available dissolved oxygen. The dissolved oxygen was on decreased from the onset with value of 4.85 mg/L to 2.40 mg/L after 7 days and 0.95 was recorded at the end of 14 days (Figure 4a). The highest reduction efficiency (80.41%) was recorded after 14 days (Figure 4b). This was as a result of activities and higher number of microorganisms during that period.

The value recorded for Total alkalinity in raw Mobi poultry slaughterhouse waste water is 143.08 mg/L (Figure 5a). This value is higher than required standard for discharge into the environment. However, after period of treatment for 7 and 14 days respectively, 125.64 and 55.84 mg/L were recorded (Figure 4.5a) at 12.19 and 60.97 % reduction efficiencies (Figure 5b).

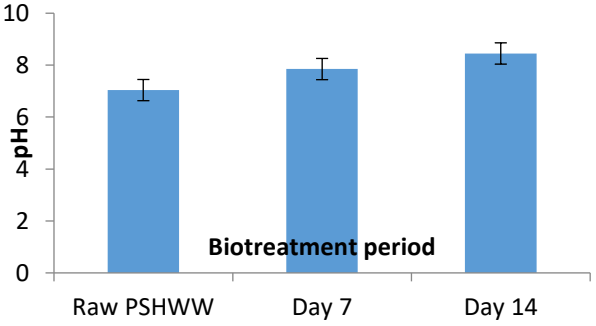

Figure 1: pH of the Bio treated Raw Poultry

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Slaughterhouse Wastewater with *Chlorella vulgaris ChA* after 7th and 14th day of treatment

Note: Raw PSHWW= Raw Poultry Slaughterhouse Wastewater

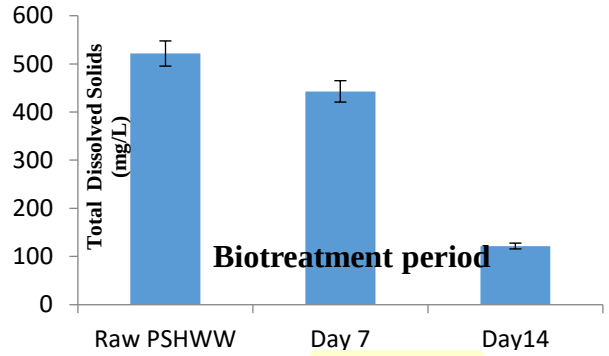

Figure 2a: Total Dissolved Solids of the Bio treated Raw Poultry Slaughterhouse Wastewater with *Chlorella vulgaris ChA* after 7th and 14th day of treatment

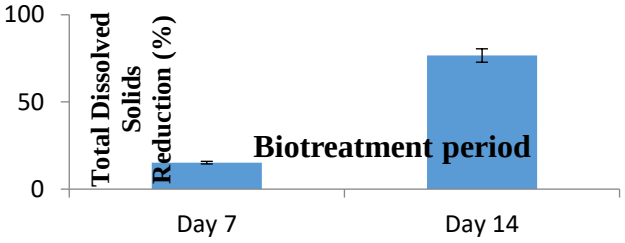

Figure 2b: Total Dissolved Solids removal efficiencies of *Chlorella vulgaris* after 7 and 14 days of treatment.

Note: Raw PSHWW= Raw Poultry Slaughterhouse Wastewater

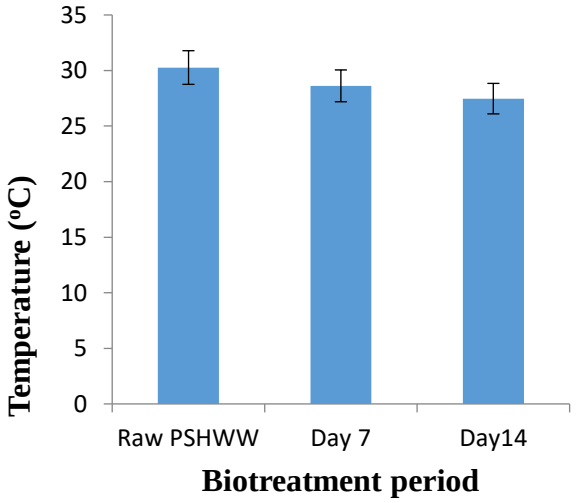

Figure 3: Temperature of the Bio treated Raw Poultry Slaughterhouse Wastewater with *Chlorella vulgaris ChA* after 7th and 14th day of treatment

Note: Raw PSHWW= Raw Poultry Slaughterhouse Wastewater

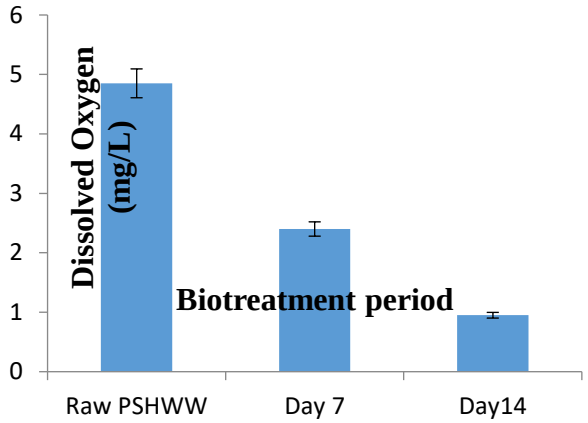

Figure 4a: Dissolved Oxygen of the Bio treated Raw Poultry Slaughterhouse Wastewater with *Chlorella vulgaris ChA* after 7th and 14th day of treatment

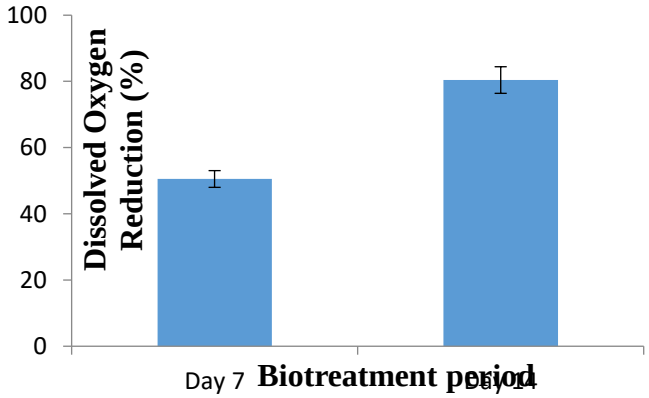

Figure 4b: Dissolved Oxygen Removal efficiencies of *Chlorella vulgaris* after 7 and 14 days of treatment

Note: Raw PSHWW= Raw Poultry Slaughterhouse Wastewater

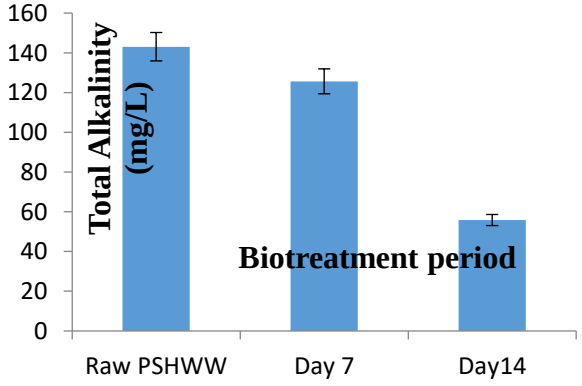

Figure 5a: Total Alkalinity of the Bio treated Raw Poultry Slaughterhouse Wastewater with *Chlorella*

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vulgaris ChA after 7th and 14th day of treatment

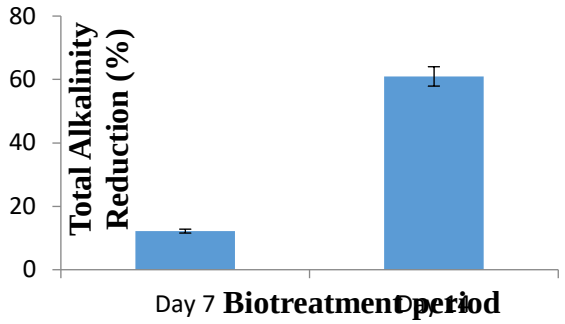

Figure 5b: Total Alkalinity Removal efficiencies of *Chlorella vulgaris* after 7 and 14 days of treatment

Note: Raw PSHWW= Raw Poultry Slaughterhouse Wastewater.

Biochemical Oxygen Demand (BOD) recorded at Mobi poultry slaughterhouse waste water was found to be lower (1125.63 and 486.81mg/L) after 7 and 14 days of bio-treatment compare with 1480.05 mg/L obtained for raw Mobi poultry slaughterhouse waste water (Figure 6a). They had reduction efficiencies of 23.95 and 67.11% after 7 and 14 days of biotreatment *Chlorella vulgaris* ChA (Figure 6b).

High degradation rate at the week two(day=14) could possibly be as a result of the acclimatization of the microorganisms to the prevailing conditions High organic material presents in the abattoir wastewater are an indication of higher BOD and COD. Higher COD and BOD concentrations recorded at Mobi poultry slaughterhouse waste water was due to high blood volume.

This is in conformity with the finding of del Pozo *et al*. (2003). This fact had a great influence on the rest of the parameters and the nature of the wastewaters. Some information on the wastewater biodegradability can be gained comparing different measures, example, BOD and COD where a high ratio of BOD to COD shows a relatively high biodegradability whereas a low ratio indicates that the wastewater is more slowly biodegraded (Vollertsen and Hvitved-Jacobsen, 2002).

The COD observed in this study showed that Mobi poultry slaughterhouse waste water reduced to 1085.10 .84 and 56 mg/L respectively from initial raw waste water value of 1778.24 mg/L after 7 and 14 days treatment with *Chlorella vulgaris* ChA (Figure 7a) at removal efficiencies of 38.98 and 96.80 % respectively (Figure 7b). The rate of reduction of COD of Mobi poultry slaughterhouse waste water confirms the effectiveness of degradation process to reduce the pollutant load contained in the wastewater.

The value recorded for ammonia in this study is 78.30 mg/L for Mobi poultry slaughterhouse waste water, however there was a decrease in result obtained after treatment for period of 14 days, with 60.85 and 33.09 mg/L at day 7 and 14 respectively (Figure 8a) as well as 22.29 and 57.74% reduction efficiencies (Figure 8b).

Phosphate and nitrate are among the prominent compounds in any abattoir effluent. The levels phosphate and nitrate compounds were higher in Mobi poultry slaughterhouse waste water. This may be attributable to the high fecal contents of the effluents. Rodier (2009) reported that wastewater samples must have less than 50 mg/l of nitrates and 0.5 mg/l of phosphate before its discharge into aquatic environment. Relatively higher decrease (12.27 and 7.83mg/L Figure 9a) with reduction efficiencies of (25.82 and 56.66 % Figure 9b at day 7 and 14 respectively) was recorded in phosphate concentration after bio-treatment against value observed for raw Mobi poultry slaughterhouse waste water 16.54 mg/L.

High phosphate levels will result in the eutrophication of the river. Blood is also the major contributor to the nitrogen content while phosphorus originates from stomach contents in the effluent.

The results obtained in this study showed significant reduction of nitrate in Mobi poultry slaughterhouse waste water after bio-treatment for period of 14 days with 11.45 and 9.30 mg/L at day 7 and 14 against 454 mg/L recorded for raw Mobi poultry slaughterhouse waste water (Figure 10a). The higher percentage reduction efficiencies were recorded after 7 and 14 days of bio-treatment (75.45 and 97.95) respectively (Figure10b).

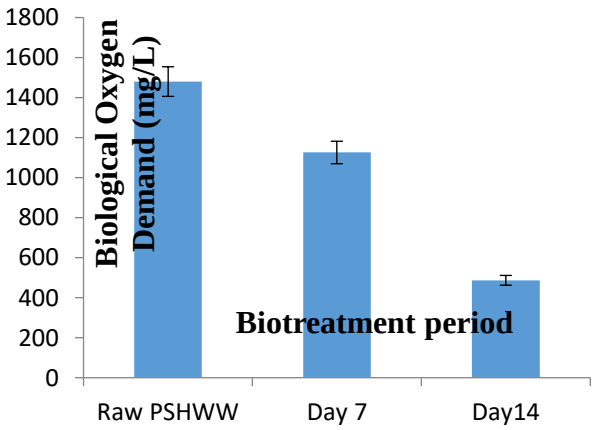

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Figure 6a: Biological Oxygen Demand of the Bio treated Raw Poultry Slaughterhouse Wastewater with *Chlorella vulgaris ChA* after 7th and 14th day of treatment

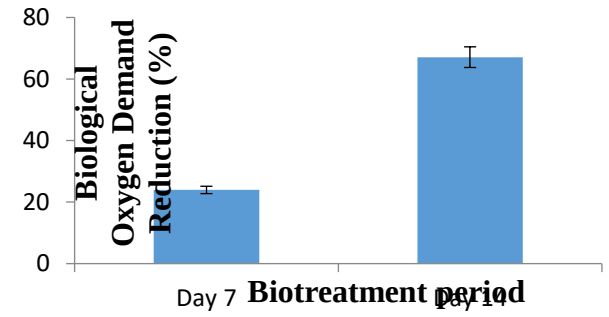

Figure 6b: Biological Oxygen Demand Removal efficiencies of *Chlorella vulgaris* after 7 and 14 days of treatment Note: Raw PSHWW= Raw Poultry Slaughterhouse Wastewater

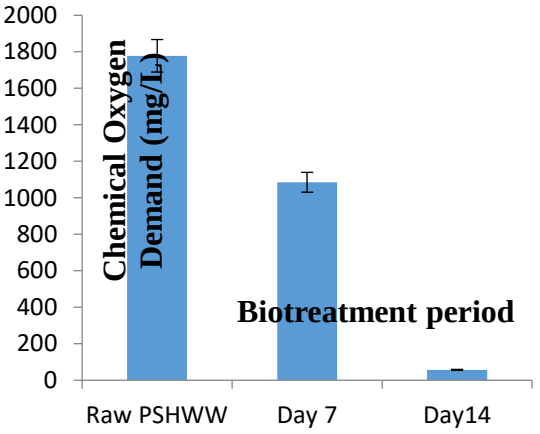

Figure 7a: Chemical Oxygen Demand of the Bio treated Raw Poultry Slaughterhouse Wastewater with *Chlorella vulgaris ChA* after 7th and 14th day of treatment

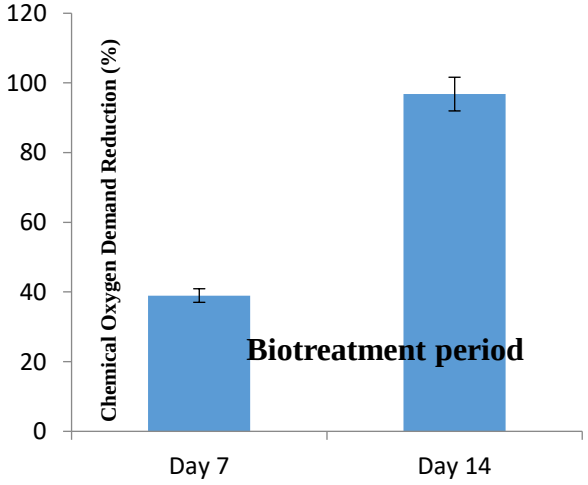

Figure 7b: Biological Oxygen Demand Removal efficiencies of *Chlorella vulgaris* after 7 and 14 days of treatment

Note: Raw PSHWW= Raw Poultry Slaughterhouse Wastewater

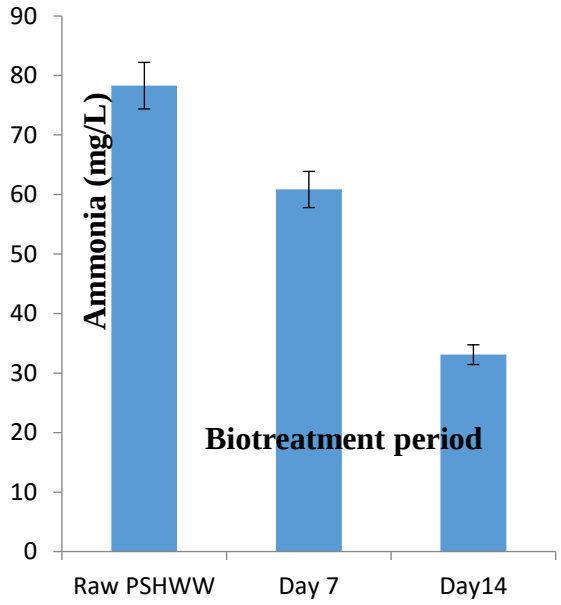

Figure 8a: Ammonia of the Bio treated Raw Poultry Slaughterhouse Wastewater with *Chlorella vulgaris ChA* after 7th and 14th day of treatment

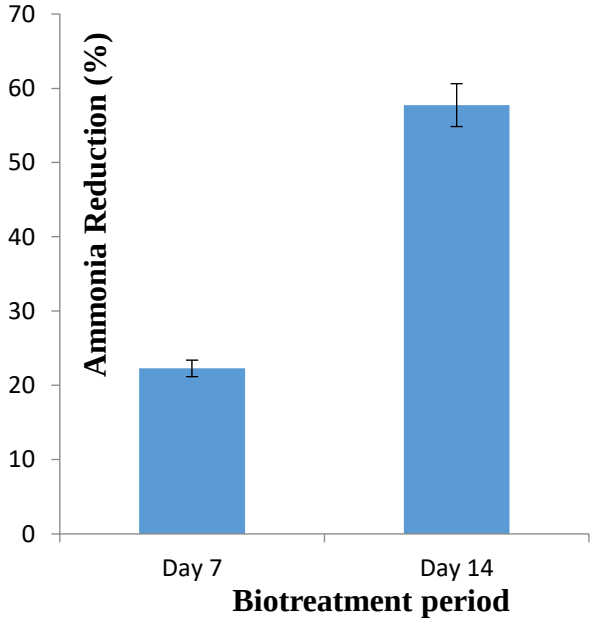

Figure 8b: Ammonia Removal efficiencies of *Chlorella vulgaris* after 7 and 14 days of treatment

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Note: Raw PSHWW= Raw Poultry Slaughterhouse Wastewater

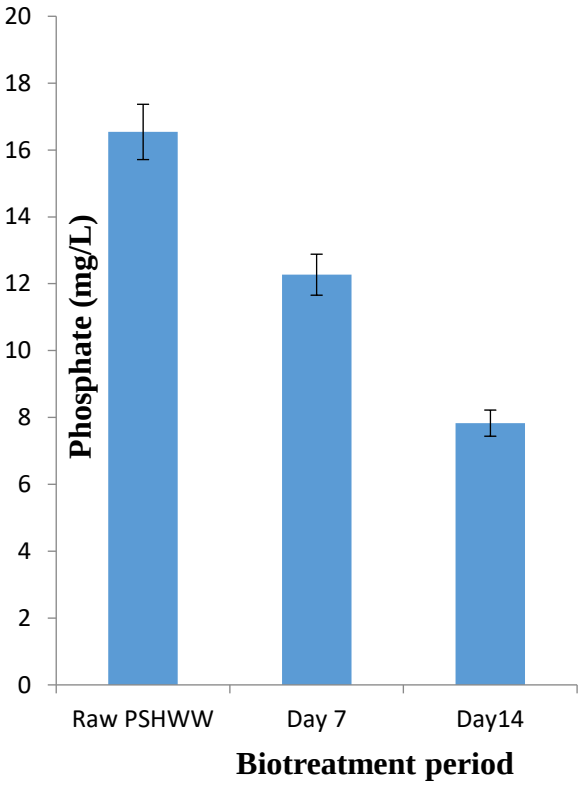

Figure 9a: Phosphate of the Bio treated Raw Poultry Slaughterhouse Wastewater with *Chlorella vulgaris ChA* after 7th and 14th day of treatment

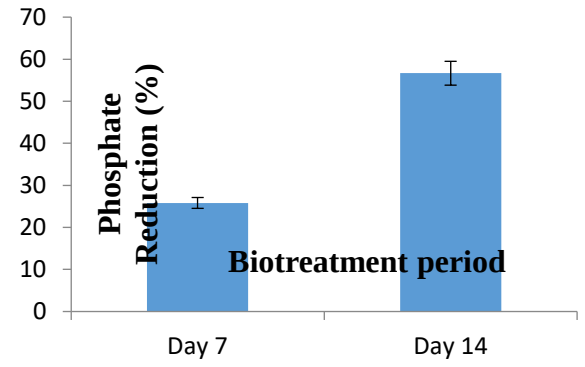

Figure 9b: Phosphate Removal efficiencies of *Chlorella vulgaris* after 7 and 14 days of treatment

Note: Raw PSHWW= Raw Poultry Slaughterhouse Wastewater

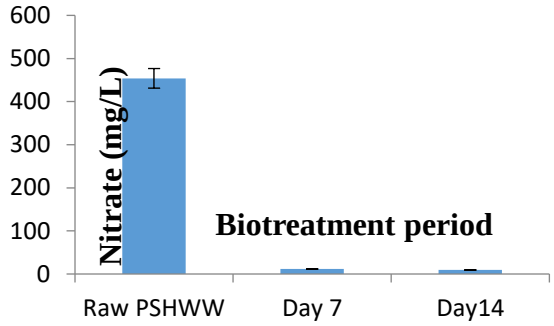

Figure 10a: Nitrate of the Bio treated Raw Poultry Slaughterhouse Wastewater with *Chlorella vulgaris ChA* after 7th and 14th day of treatment

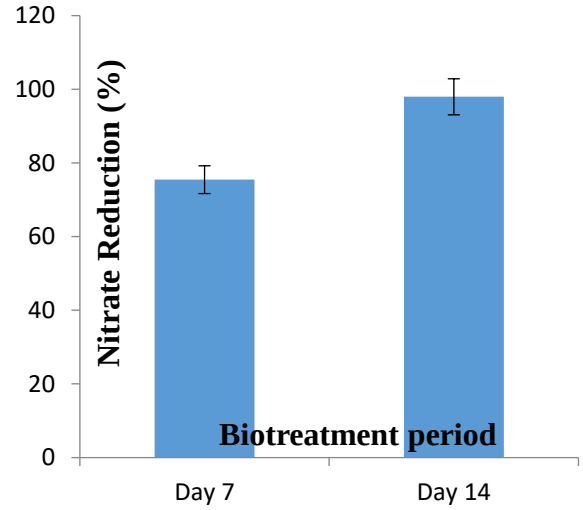

Figure 10b: Nitrate Removal efficiencies of *Chlorella vulgaris* after 7 and 14 days of treatment

Note: Raw PSHWW= Raw Poultry Slaughterhouse Wastewater

IV. CONCLUSION AND RECOMMENDATION

Raw poultry slaughterhouse waste water from Mobi area in Ede South Local Government was successfully bio-treated with Microalgae, the values of physicochemical parameters obtained for the raw poultry slaughterhouse waste water in this study were reduced to acceptable standard for discharge to environment after 7 and 14 days of treatment

CONCLUSION

Poultry Slaughterhouses releases effluents that are heavily loaded with various form organic matter that are hazardous to environment. The discharge of this waste into the immediate environment, waterways and aquatic environment without proper treatment impacts on the water quality. The physicochemical analysis of the

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poultry slaughterhouse wastewater in this study do not meet National Environmental Standards and Regulation Enforcement Agency (NESREA) permissible limit, and therefore not suitable for discharge into environment. It is therefore essential to adopt appropriate slaughterhouse wastewater treatment measures to prevent the contamination of the environment including surface water and ground water. This study inundates the fact that untreated abattoir effluents generated at the mobi poultry slaughterhouse waste water constitute serious environmental problem to the abattoir neighborhood and health problem to people within the area, hence there should be an enforcement of strict environmental management by regulatory authorities.

RECOMMENDATION

Given none of the studied slaughterhouses can reach the desirable level of standards, to improve the quality of slaughterhouse wastewater and use it for agriculture purposes following items are recommended: minimizing and recycling byproducts, separation and collection of blood and gastric contents from raw wastewater and use in industry such as pharmaceutical industry, collecting fat from raw wastewater and using them in Soap industry, biological and chemical treatment of slaughterhouse wastewater.

Finally, the slaughterhouses are normally controlled by local bodies, which should follow the standards prescribed, but due to non-existence of modernized slaughterhouses, environmental pollution arising out of the slaughtering activities cannot be controlled. It is suggested that the local bodies take up modernization of slaughterhouses and achieve the pollution control norms.

APPENDIX

Appendix 1: 18SrRNA sequence of *Chlorella vulgaris* strain SAG 30.80 with 99% of identity

AGATGAACTTGCGGAAGGATCATTGAATCTAT
CGAATCCACTTTGGTAACCACTCGTCCCCCTCG
TCCGATGTCGCCCTCTCTTAGGGGAGTGCGAT
GCGGCGAGCGTCGGTCCCCTGGCTGTGGCTCC
CCGAGCTGTTGCTCAGGTCCGGCGGGCGTCCC
TTCACATGTGGGACCCCTTCTTTTTGAGGGGAC
CACCCCTTTGGAGGATCCGACGTCGGAAATTT
CAACTCAACTCACCCACCCCAAACTGAAACTT
ATTCTAAAGCACCTTGTGGTTGGCAGCTCGTCT
GCCGTCCACTCCAAACCAAAACAACTCTCAAC
AACGGATATCTTGGCTCCCGTATCGATGAAGA
ACGCAGCGAAATGCGATACGTAGTGTGAATTG
CAGAATTCCGTGAACCTCGAATCTTTGAACGC
AACTTGCGCCTGAGGCTTCGGCCAAAGGCATG
TCTGCCTCAGCGTCGGCTCACCCCCTGCCTCCC
CATCTCCTTTGATTGGGAAGGCGGATCTGACCT
TCCCGGTTCCGCCGGTCACTCGTGATTGGCGCC
GGGTCGGTTGAAGCTCAGAGGTATGAGCATGG
ACCCCGTTCGCAGGGTAATGGCTTGGTAGGTA
GGCATTCCCTACGCATCCTGCCGTTGCCCGAG
GGGACTTTGCTGGAGACCTAGCAGGAATTCGG
ATGCTTGGGCACCCCCCGACACCGAAACTCTT
CATTTCGACCTGAGCTCAGGCAGACTACCCGC
TGTAGGTT

ACKNOWLEDGEMENTS

We are grateful to the Almighty for His divine guidance, protection, wisdom and for successful completion of this work.

REFERENCES

[1] N. Abdel Rand, N., Al-Homaidan, A.A. & Ibraheem, B.M.. Macroalgae and wastewater treatment. *Journal of Biological Science*, 2012, 19(3):257-275.

[2] Dereeper, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res.2008 1:36.

[3] R.Edgar RC: MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004, 32(5):1792-1797.

[4] G. Talavera., and Castresana, J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology, 2007 56, 564-577.

[5] M.W.Akan., Paramasivam, M. and Ganguly, M. Assessment and occurrence of various heavy metals in surface water of Ganga River around Kolkata: A study for toxicity and ecological impact. *Environmental monitoring and assessment* , 2010, 160: 207- 213.

[6] O.B. Akpor, and Muchie, M. Challenges in Meeting the MDGs: The Nigerian Drinking Water Supply and Distribution Sector. Journal of Environmental Science and Technology, 2011 4: 480-489.

[7] M. Radojevic, Bashkin VN. Practical environmental analysis. Royal school of chemistry, Thomas Graham house, science park Cambridge, UK., 1999 645 p

[8] E. Padilla-Gasca Lopez-Lopez A, Gallardo-Vaidez J. Evaluation of Stability Factor in the Anaerobic Treatment of Slaughterhouse Wastewater. J. Bioremed. Biodegrad., 2011, 2:114.

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[9] J.Vollertsen, Hvitved-Jacobsen T. Biodegradability of wastewater – a method for COD-fractionation. Water Sci. Technol., 2002 45(3):25-34.

[10] R.Del Pozo, Tas DO, Dulkadiroglu H, Orhon D, Diez V. Biodegradability of slaughterhouse wastewater with high blood content under anaerobic and aerobic conditions. J. Chem. Technol. Biotechnol, 2003.78:384-391

[11] A.O. Adesemoye,., Opere, B.O. and Makinde, S.C.O. Microbial content of abattoir wastewater and its contaminated soil in Lagos, Nigeria. Afr. J. Biotechnol., 2006 5 (20): 1963-1968

[12] G.Valladão, M. R., Pádua, S. B., Gallani, S. U., Menezes-Filho, R. N., Dias-Neto, J., Martins, M. L. and Pilarski, F.. *Paratrichodina africana* (Ciliophora): a pathogenic gill parasite in farmed Nile tilapia. *Veterinary Parasitology*, in press., 2007 doi: 10.1016/j.vetpar.2013.04.043

[13] American Public Health Association (APHA),. "Standard method for examination of water and waste water", 20th Edition. *American Public Health Association*,Washington DC *Anaerobia*. Sao Paulo, Brasil, 1988. 363-36.

[14] AOAC. Official Methods of Analysis. 18th Edition, Association of Official Analytical Chemists, Gaithersburgs, MD., 2006

[15] APHA, (16th edition), Standard Methods for the Examination of Water and Wastewater, 1985, p. 1268.

[16] B. Austin,, and Austin D.A. Bacterial Fish Pathogens.3rd edition. Chichester, UK: Springer Praxis, 1999

[17] G.Bitton,.Wastewater Microbiology. 3rd edition.New Jersey: John Wiley & sons, incorporation, 2005

[18] C.F. Bustillo-Lecompte, & Mehrvar, M. Treatment of an actual slaughterhouse wastewater by integration of biological and advanced oxidation processes: modeling, optimization, and cost-effectiveness analysis. *Journal of Environmental Management*,2016, 182(1): 651-666.

[19] M.C. Cammarota. & Freire, D.M.G. A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresource Technology*,2006, 97(17):2195-2210.

[20] S.Chinnasamy, A. Bhatnagar, R.W., Hunt and K.C. Das,. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresource Technol.*,2010 101: 3097-3105.

[21] L. De-Bashan, and Bashan, Y. Immobilized microalgae for removing pollutants: Review of practical aspects. *Bioresource Technology*, 2010 101: 1611- 1627

[22] R.P.Dinrifo, Babatunde SOE, Bankole YO, Demu QA. PhysicoChemical Properties of Rain Water Collected from Some Industrial Areas of Lagos State Nigeria. European Journal of Scientific Research, EuroJournals Publishing, Inc, pp, 2010, 383-390

[23] N, Edema., Okoloko, G. and Agbogidi, M. Physicochemical Characteristics of the Water Soluble Fraction of Ogini well head crude oil and the effects on Pistia stratiotes Linn. J.Agric. Environ. Sci, 2001, 2(6):633-638

[24] Godos, Blanco, S., García-Encina, P.A., Becares, E. and Munozm, R. Long-term operation of high rate algal ponds for the bioremediation of piggery wastewaters at high loading rates. *Bioresource Technology*, 2003, 100: 4332-4339

[25] D. Massé & L. Masse. Characterization of wastewater. *Canadian Agricultural Engineering*,2000 42(3):139-146.

[26] W. Oswald, and C.G. Golueke. Biological transformation of solar energy. *Adv. Applied Microbiology*. 1960 2: 223-262. PMID: 13731568

[27] S. Radie, Stipanicev, D., Cvjetko, P., Marijanovic Rajcic, M., Širac, S., Pevalek-Kozlina, B. and Pavlica, M. Duckweed Lemna minor as a tool for testing toxicity and genotoxicity of surface waters, *Ecotoxicology and environmental safety*, 2009, 74: 182–187.

[28] E. Rigo, Rigoni, Lodea, P., De Oliveira, D., Freire, D. M., Treiche, L.H. & DI Luccio, M. 2008. Comparison of two lipases in the hydrolysis of oil and grease in wastewater of the swine meat industry. *Industrial & Engineering Chemistry Research*, 47(6):1760-1765.

[29] D. Tanikawa,,Yokote, N., Nakahara, K. & Yamaguchi, T. Evaluation of Process Performance for Lipid-rich Wastewater Treatment Using a Combination System of an Anaerobic Baffled Reactor and an Aerobic Trickling Filter. *Journal of Water and Environment Technology*,2016 14(2):90-95.

[30] E. Zhang, Wang, B., Wang, Q., Zhang, S., and B. Zhao,. "Ammonia-nitrogen and orthophosphate removal by immobilized *Scenedesmus sp.* isolated from municipal wastewater for potential use in tertiary treatment". *Bioresource Technology*, 2008 99: 3787 - 3793.

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