Optimum conditions for Cultivation of *Chlorella vulgaris* on Oil Palm Residue Extracts

Abstract

The need to manage agro waste in the environment has continued to generate worries to most developing countries. Oil palm mill waste cause pollutions of soil, water and environment but could be exploited as substrate for algal growth because of its rich mineral and carbohydrate contents. In this study Oil palm cake and Empty fruit bunch residue were obtained from Aluu, Rivers State Nigeria. The proximate, physicochemical analysis of the extract were ascertained, wavelength selection of extract was done to monitor for biomass production, growth media ratio of extract and One Factor at a Time (OFAT) was employed for the optimization of *Chlorella vulgaris* growth on the Oil Palm Residue Extracts. The proximate result revealed that the range of Ash (8.40 ± 0.30 - 6.60 ± 0.40 ppm), Moisture (10.90 ± 0.30 - 6.75 ± 0.65 ppm), Lipid (22.05 ± 0.35 - 14.90 ± 0.30 ppm), Protein (1.30 ± 0.18 - 0.80 ± 0.08 ppm), Fiber (25.50 ± 0.50 - 39.09 ± 0.57 ppm) and Carbohydrate (29.83 ± 0.29 - 29.29 ± 0.58 ppm). The physicochemical composition pH (5.15 ± 0.02 - 5.36 ± 0.06), Conductivity (224.15 ± 0.45 - 481.00 ± 2.00 µS/cm), DO (2.87 ± 0.06 - 4.86 ± 0.06 ppm), Nitrate (31.05 ± 0.48 - 40.33 ± 0.98 ppm), Sulphate (36.35 ± 0.46 - 50.68 ± 0.49 ppm) and phosphate (27.71 ± 0.50 - 37.35 ± 0.55ppm) were obtained for Oil palm cake and Empty fruit bunch respectively. wavelength of 620 nm exhibited better accuracy for the biomass monitoring and optimization investigations. Growth media ratio selected was 180:20 for empty fruit bunch: oil palm cake. The optimization revealed pH 6.0, temperature 30^{o}C, salinity 10ppm and photoperiod 12:12 day: night as optimal condition for the growth of *Chlorella vulgaris* biomass. The oil palm residue can be a veritable medium for growth of this algae and biotechnological application in waste management.

Keywords: Oil palm cake, Oil palm empty fruit bunch, *Chlorella vulgaris*,

1.0 Introduction

The main source of palm oil is the African oil palm '*Elaeis guineensis*' (*Elaeis* (from Greek, meaning "oil"), the specie name *guineensis* alluding to its country of origin Guinea) [1]. The palm kernel press fiber (PPF), nut, and palm kernel shell (PKS), are the processing intermediates from oil palm's fresh fruit bunch (FFB). The by-products include decanter cake, empty fruit bunch chaff, palm kernel cake, palm pressed fiber, and palm oil mill effluent. Empty fruit bunches are a plentiful source of fibrous material with a wholly organic origin in a conventional palm oil mill. It is one of the lignocellulosic materials that is very important since the oil palm industry produces a significant amount of biomass [2]. Empty fruit bunches are seen as unwanted

due to the expense of storing, transporting, distributing, and treating them. Palm kernel cake is an agro-industrial by-product of oil palm mill. It is the solid residue left behind after mechanical expelling/ solvent extraction of oil from the kernels of the palm fruits. It is now well entrenched as a major feed ingredient in beef and dairy feed because it can serve as a feed formulation in order to increase the weight of fully-grown animals [3]. The PKC is obtained out from two stages of oil extraction from the palm fruit. The first stage is the primary extraction of palm oil from the pericarp portion of the fruit, which also produces the kernel and by-products Palm Oil Sludge and Palm Pressed Fibre. The extraction of oil from crushed kernel then results in the production of PKC as a by-product. During the last century, a great deal of study and advance, as well as applications, has been devoted to waste. These include waste minimization and treatment, environmental assessment of waste, minimization of environmental impact, life cycle assessment, phycology and biotechnology, and others [4]. The major reason for such huge efforts is that waste cohort constitutes one of the foremost environmental problems where production industries are concerned. Until now, an accumulative pressure has been put on discovery methods of recycling waste, for instance through cleaner production, thus mirroring rapid changes in environmental policies [5]. Production of huge amounts of crude palm oil results in larger amounts of palm oil mill waste. It is worth considering the potential value of oil palm waste prior to its treatment through introduction of a cleaner production. Microalgae can be described as a simple unicellular photosynthetic organism that uses energy from sunlight and carbon dioxide from the atmosphere. Microalgae have been identified as an operative tool to sequester solar radiation and yield huge biomass with little or no harm on the ecosystem [6]. They are cultivated in medium with reduced nitrogen and phosphorous elements. The metabolism of microalgae allows lipid and carbohydrate to be synthesized using biomass because of their vital properties [7]. Utilization of the microalga *Chlorella* sp has proved effective due to its high-valued potential substances such as chlorophyll, carotene and protein amongst others, [8] and due to their tolerance levels for the high concentration of CO_2 and high proficiency in utilizing CO_2 during photosynthesis [9]. *Chlorella* sp. is among the fastest growing photosynthetic organisms having carbon fixation rates in order of magnitude higher than those of land plants. It utilizes CO_2 as one of its main building blocks and is a practical option for anthropogenic CO_2 capture and sequestration [10]. There are three biotechnology field concerned with the application of microalgae; Red biotechnology field which is the most feasible biotechnology because it concerns medicine and health application such as drugs, bioactive substances and neutraceuticals. Green biotechnology field concern with agriculture, water and environment application such as feed for herbivorous livestock, food for human and reduction in greenhouse gases emitted. White biotechnology field also concerns with an industrial application such as biofuel production [11]. The objective of this study is the cultivation of green microalgae *Chlorella vulgaris* on low-cost carbon substrate; oil palm cake (palm kernel cake) and oil palm empty fruit bunches.

2.0 Materials/Method

2.1 Collection of the oil palm waste

A sterile bag was used to collect the oil palm empty fruit bunch and oil palm cake from a palm oil mill in Aluu village in Port Harcourt, Rivers State. The oil palm empty fruit bunch and palm

oil cake were properly dried to remove moisture and carefully grounded thoroughly into a finely powdered form and then transported to the laboratory.

2.2 Lighting

The lighting source was natural light from sunlight. The cultures were arranged on laboratory benches from where they received sunlight through the window.

2.3 Washing and sterilization

All glasswares were washed and sterilized in hot air oven at 160 ^{0}C for 60 minutes and media used were also sterilized by autoclaving at 15 psi and 121^{0}C for 15 minutes.

2.4 Assay of physicochemical properties of the extract

The samples' physicochemical characteristics were assessed using the [12] standard analytical process. When the samples arrived at the laboratory, the following physiochemical parameters were examined: Total dissolved solids (TDS), conductivity, salinity, pH, chloride, nitrate, phosphate, sulfate, ammonia, Dissolved oxygen (DO), Biochemical Oxygen Demand (BOD), and Chemical Oxygen Demand (COD)

2.4.1 pH Determination

After standardizing and calibrating the pH meter (Orion star A211) using buffers 4, 7, and 10, the pH of the water samples was determined. In order to prevent an electrode from cracking, the electrode had to be put into the extract with its bulb being sure not to touch the bottom of the sample container. After that, pH readings were taken.

2.4.2 Chlorides
Method: Titration method

Procedure: Silver nitrate titration was used to assess the sample's chloride content. Five milliliters of the material were mixed with a few drops of potassium chromate. It was then titrated with silver nitrate (AgNO3). After the color transition from yellow to the end-point (orange), the tire value was noted.

2.4.3 Salinity
Method: Titration method

Procedure: The chloride concentration in the sample was determined by silver nitrate titration. A few drops of potassium chromate were added to five milliliters of the sample. After a color change to yellow, it was then titrated with silver nitrate ($AgNO_3$). The tire value was recorded after a colour change from yellow to the end-point (orange).

2.4.4 Nitrate
Method: Bruccine colorimetric method

Procedure: By adding 0.5 ml of Brucine solution to 1 ml of the sample in a clean test tube, the nitrate content was calculated using the Bruccine colorimetric method. Sulphuric acid (H_2SO_4)

concentration of 2 ml was added. This was homogenized and let to stand for 30 minutes. Then, 2 ml of distilled water was added. After 15 to 30 minutes, the absorbance was measured using a UV- 4 Unicam spectrophotometer at a wavelength of 740 nm.

2.4.5 Phosphate
Method: Ammonium Molybdate method

Procedure: A 50 ml sample of the extract was measured into a 500 ml container with H_2SO_4 (37% v/v) and 5g of ammonium sulphate, and the container was thoroughly mixed. Then it was cooled and rinsed with distilled water after being heated on a hot plate (30^oC) until the volume was less than 30 ml. Sodium hydroxide was used to make up to 400 ml, and about 4 drops of the phenolphthalein indicator were also added. After carefully mixing, an additional 5 ml of 37% H_2SO_4, 5ml of ammonium molybdate, and 5ml of amino solution were added. The solution's absorbance was measured using UV- 4 spectrophotometers at 650 nm.

2.4.6 Sulphate
Method: Turbidometric method

Procedure: A tablespoon of barium chloride crystals was added, and the mixture of 4 ml of buffer solution with 20 ml of sample was mixed for 60 seconds. The UV-4 unicam spectrophotometer was used to quantify turbidity at a wavelength of 470 nm.

2.4.7 Ammonia
Method: Distillation and titration method

Procedure: A distillation flask was filled with around 50ml of the extract, which was then adjusted to a pH of 7.0. 50ml of distilled water and 250ml of sodium hydroxide solution were combined. To ensure steady boiling and prevent flask splitting, a small amount of zinc granules was added. Boric acid (100ml), methyl red (2 drops), and bromocresol green (2 drops) were added as indicators. The mixture was added to a distillation column and titrated against 0.20 M NH_4Cl, which caused the mixture to turn pink from green.

2.4.8 Determination of Conductivity
The conductivity of the extract was measured using an electronic conductivity meter. According to the manufacturer's instructions, the probe's sensitive section was lowered into a solution of potassium chloride (KCl) for 15 minutes to pre-calibrate it. By submerging the sample in a water bath to reach the necessary temperature of 25^oC. The bridge balance and resistance were displayed on the equipment's LCD whilst the electrode was submerged in the sample.

2.4.9 Biochemical Oxygen Demand (BOD)
This was accomplished by pipetting 50ml of the extract into a 200ml BOD bottle, which was then filled with 150ml of distilled water). The bottle was completely filled and then sealed without retaining any air bubbles. Using the blank dilution water, the initial DO in the sample after 15 minutes at room temperature was calculated. The sample was then incubated at room temperature for 5 days. The amount of dissolved oxygen that was still present in the incubated samples after 5 days was calculated.

The BOD_5 was determined as $BOD_5 = DO_0 - DO_5$ (eq 2.1)

Where DO_0 and DO_5 is initial dissolved oxygen and dissolved oxygen at the 5th day respectively.

2.4.10 Chemical Oxygen Demand (COD)

25 milliliters of the sample were added to a 250 mls conical flask along with 10 milliliters of diluted H_2SO_4 and 10 mls of 0.0125N $KMNO_4$ (potassium permanganate). The mixture was thoroughly mixed, and it bred for four hours at room temperature. When the purple to pink color of the permanganate tried to vanish, 10ml of $KMNO_4$ was added to retain a clear excess. The mixture was checked periodically. The same amount of the reagent used for the sample was added to 250 ml of distilled water, which was then incubated at room temperature for 4 hours to create a blank. After the 4 hours of incubation, 1ml of 10% potassium iodide (KI) was added, and the resulting solution was titrated using starch as an indicator with 0.0125M sodium thiosulphate. The titration continues until the blue color has vanished to leave behind a colorless substance. The blank was titrated once more.

2.5 Proximate Analysis of Empty Fruit Bunch and Oil palm cake

The nutritional value of the empty fruit bunch was determined through physical and chemical analysis of the oil palm empty fruit bunch. The [12] test technique was used for the analysis that follows.

2.5.1 Moisture Content

By drying around 30g of the sample to a constant weight in a crucible inside a hot air oven set at 120°C, the sample's moisture content was calculated gravimetrically. The final weight was recorded after desiccators were used to cool the sample to room temperature.

$$\text{Moisture}(\%) = \frac{WF - WD}{WU} \times \frac{100}{1} \qquad \text{(eq 2.2)}$$

where WF is weight of fresh sample, WD is weight of dried sample and WU is weight of sample used.

2.5.2 Ash content

Using a clean, pre-weighed crucible and around 30g of the sample, the ash content of the sample was determined gravimetrically. On a plate, this was slowly heated to complete carbonization. After that, it spent 24 hours at 550°C in a furnace where white or grey ash was obtained. The cooling crucible was placed in desiccators, and the weight of the ash was noted.

$$\text{Ash}(\%) = \frac{WA - WC}{WS} \times \frac{100}{1} \qquad \text{(eq 2.3)}$$

where WA is weight of crucible and ash sample, WC is weight of crucible and WS is weight of sample.

2.5.3 Protein Content

The approach chosen was Kjeldahl. Digestion is the first step in the procedure: A clean, 250 ml conical flask with a weight capacity of 0.1 palm kernel chaff was filled with the material. Three grams of a digestive catalyst were also added, and the palm kernel chaff was heated to cause the material to change color from black to sky blue. The digest was diluted with distilled water to a

volume of 100ml after being chilled to room temperature. Distillation is done in the second stage. A distillation flask was filled with 20 ml of diluted digest and placed on the hot plate or electrothermal heater while being held in place. The receiver contained 10 mls of 2% boric acid indicator, and the Liebig condenser to which the distillation flask was attached. The digest received a 40mls infusion of 40% sodium hydroxide, which significantly increased its alkalinity. The mixture was heated to boiling before adding the boric acid, which caused the ammonia distillate to turn from purple to greenish. In the third phase, the distillate was titrated with a standard O.1N hydrochloric acid solution to change it from greenish to purple again. Titer number was recorded in order to keep track of how much hydrochloric acid was administered to effect this change.

$$\text{Organic Nitrogen (\%)} = \frac{\text{Titre value} \times 1.4 \times 100 \times 100}{1000 \times 20 \times 0.1} \quad \text{(eq 2.4)}$$

Calculation % Organic Nitrogen = (Titer value x 1.4 x 100 x 100)/1000x20x0.1

where

Titre value is the volume of HCL used in titrating the ammonium distillate, 1.4 is nitrogen equivalent to the normality of HCL used in the nitration 0.1N, 100 is the total volume of digest dilution, 100 is the percentage factor, 1000 is the conversion factor from gram to milligram, 20 is the integral volume of digits analyzed or distilled and 0.1 is the weight of sample in gram digested.

2.5.4 Crude Fibre

Acid-alkaline hydrolysis was employed in this process. In order to do this, 2g of the sample must be boiled in a beaker with 0.1 MF12804 and 0.1 M NaOH. The mixture must then be filtered through a Buchner funnel, dried, and ashed at 550°C.

2.5.5 Crude Lipid

About 2g of the sample was placed on a filter paper, which was then placed into a Soxhlet extractor and then into a dried distillation flask that had been pre-weighed. Acetone was then injected into the distillation flask via the condenser end attached to the extractor. The heated solvent chamber was extracted by continuous refluxing using the condenser connected to coolant with a constant supply of cold water until the lipid was clearly extracted entirely from the sample. The solvent was evaporated in order to concentrate the lipid. By weighing the flask after it had been dried to a fixed weight, the percentage of lipid was calculated.

$$\text{Lipid (\%)} = \frac{WFE - WEF}{WSE} \times \frac{100}{1} \quad \text{(eq 2.5)}$$

where WFE is the weight of flask and extract, WEF is the weight of empty flask and WSE is the weight of sample extracted.

2.5.6 Carbohydrate Content

Cleg Anthrone method was used for the analysis using a weight balance 0.1g of palm kernel chaff was weighted into a 25ml volumetric flask, 1ml distilled water and 1.3 ml of 62% perchloric acid was added and shake for a period of 20 minutes to homogenize completely. The flask was made up to 25 ml mark with distilled water and stopper. The solution formed was

filtered through a glass filter paper or allowed to sediment and decanted. One milliliter (1ml) of the filtrate was collected and transferred into a 10ml test tube and diluted with distilled water. One millilitre (1ml) of working solution was pipetted into a clean test tube and 5ml anthrone reagent was mixed similarly and the whole mixture was read at 630 nm wavelength using the 1ml distilled water and the 5ml anthrone reagent prepared as blank. Solution glucose of 0.1ml was also prepared and was treated as the sample with anthrone reagent. The absorbance of the standard glucose was read and the value of carbohydrate as glucose was calculated using the formula below.

$$\text{CHO as glucose } (\%) = \frac{25 \times AB}{SG} \qquad \text{(eq 2.6)}$$

AB is absorbance of sample and SG is absorbance of standard glucose.

2.6 Microbiological Analyses

2.6.1. Sample Collection

Microalgae was collected from fresh water containing the microalgae and it was transported to the Microbiology laboratory for immediate analysis.

2.6.2. Isolation of microalgae

A novel synthetic medium (Potassium nitrate (0.132 g/l), sodium silicate (0.066 g/l), monosodium phosphate (0.066 g/l), Ethylenediaminetetraacetic acid (0.066 g/l), water (1 litre), pH (7.5) according to [6] containing antibiotics (Tetracyclin and Nystatin to eliminate bacteria and fungi growth respectively), was used to isolate *Chlorella* species. Agar plate technique was used for isolation of the culture plates and incubated for three to five days in a shade under natural illumination (sunlight). Colonies which appeared after four days were sub-cultured for monoculture development. The colonies were identified using cultural and morphological characteristics.

2.7 Microalgae Identification and Harvesting

An aliquot of the isolate was taken and placed under the x40 objective of the microscope to identify algae based on the morphological characteristics using an algae atlas as a guide for similarity check, colour, size, shape and cell wall. some other putative signatures were used in the identification [13].

About two to five (2-5ml) of sterile water was poured to a mono-growth of algae cells in the culture plates and a sterile wire loop was used to dislodge from the medium. They were aseptically pipetted into a sterile conical flask and cotton plugged. This was stored in a refrigerator at 4^{o}C until further used.

2.8 Determination of optimal wavelength

By using the spectrophotometer to scan the oil palm extract from a low to a high wavelength, the ideal wavelength of the medium was discovered. The point of least absorbance was used to identify the ideal wavelength. [14] and [15]

2.9 Optical Density

Five milliliters of each sample were taken using a 5ml syringe after which 3ml of the collected sample was dispensed into spectrophotometer cuvette and the optical density determined using a spectrophotometer set at the wavelength of 620 nm. After 24 hours this exercise was repeated and subsequently for 7 days during blooming.

2.10 Process optimization (Light and Dark phases) for selection of biomass production.

The substrate ratio of the empty fruit bunch and oil palm cake was used to ascertain the best ratio of the two. While the novel synthetic media was used as positive controls and the un-inoculated ratio of the extract was used as negative controls.

2.11 Interaction of growth factors for the growth of *Chlorella vulgaris*

The method of [16] was used to detect optimal points by the sigmoid graphs to determine the interaction between the microalga, oil palm extracts and operational factors such as pH, temperature, salinity and photoperiod (12:12 and 6:18).

2.12 Biomass dry weight measurement

10mls of growing culture was centrifuged at 3000 rpm for 15 mins in other to have the dry weight. The cells were washed 3 times with physiological saline and dried at 50 ºC in a hot air oven until a constant weight was obtained with a RAGWAG AS/22 weighing balance. Then brought to room temperature in a desiccator, then the net dry cell weight was determined by measuring the arithmetic difference of final weight of the filter paper and the initial weight

3.0 RESULTS AND DISCUSSION

3.1 Proximate composition of oil palm cake, empty fruit bunch and algae biomass

Table 3.A depicts the findings for proximate composition of Oil Palm Cake (OPC), Empty Fruit Bunch (EFB) and algae biomass used for this study.

Table 3.A: Proximate composition of oil palm cake, empty fruit bunch and algae biomass

Parameter	Oil palm cake (ppm)	Empty fruit bunch (ppm)	Algae biomass (ppm)
Ash	8.40± 0.30	6.60±0.40	6.95±0.70
Moisture	10.90±0.30	6.75±0.65	5.0±0.70
Lipid	22.05±0.35	14.90±0.30	11.30±1.20
Protein	1.30±0.18	0.80±0.08	1.15±0.06
Fiber	25.50±0.50	39.09±0.57	14.52±0.49
Carbohydrate	29.83±0.29	29.29±0.58	20.52±0.49

The data obtained for physicochemical characteristics of oil palm extract (oil palm cake, empty fruit bunch) is reported on Table 3.B.

Table 3.B: Physiochemical composition of oil palm cake, empty fruit bunch

Parameter	Oil palm cake	Empty fruit bunch
pH	5.15± 0.02	5.36±0.06
EC (µS/cm)	224.15±0.45	481.00±2.00
Nitrate (ppm)	31.05±0.48	40.33±0.98
Sulphate (ppm)	36.35±0.46	50.68±0.49
Phosphate (ppm)	27.71±0.50	37.35±0.55
BOD (ppm)	0.70±0.02	0.33±0.01
COD (ppm)	77.00±1.00	128.00±2.00
TDS (ppm)	149.22±0.52	326.50±4.50
DO (ppm)	2.87±0.06	4.86±0.06
Calcium (ppm)	68.80±0.59	69.13±1.01
Magnesium (ppm)	92.51±1.04	133.94±1.53
Ammonia (ppm)	23.96±0.52	29.93±0.42
Salinity (ppm)	36.75±0.59	31.29±0.94

3.2: Wavelength for monitoring growth of Oil Palm Cake, Empty Fruit Bunch and control.

Figure 3.A denotes the response of varying wavelength and optical densities. The results determine a corresponding fall in the absorbance of the formulations as the wavelength increased. The wavelength was increased manually from 500-800nm, the corresponding absorbance level fell from 0.9nm to 0.1nm. wavelength of 620 nm exhibited better accuracy for the biomass monitoring and optimization investigations.

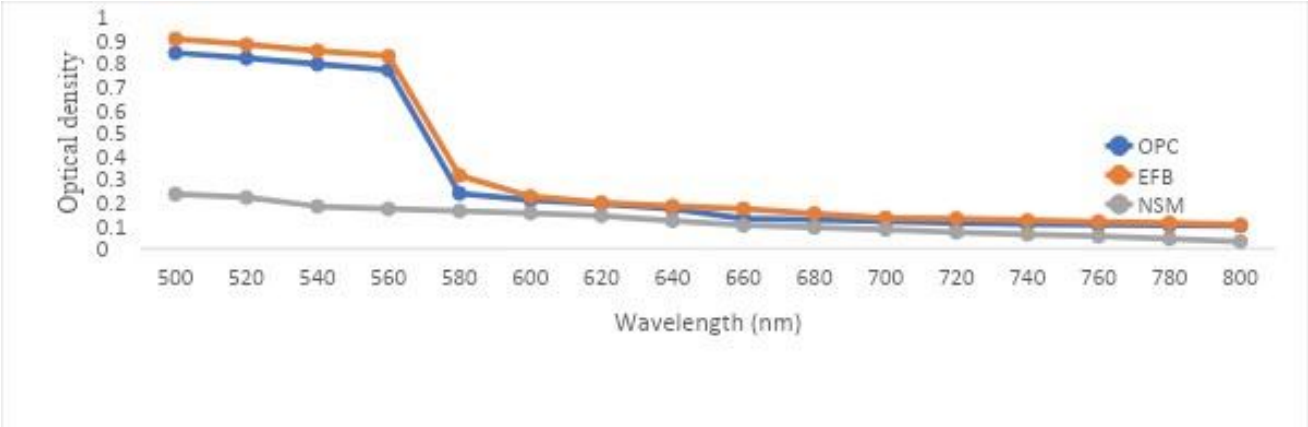

Fig 3.A: Wavelength selection for the Oil Palm Cake, Empty Fruit Bunch and

control.(NSM---Novel Synthetic Medium as control)

3.3: Growth of *Chlorella vulgaris* in different ratios of Empty Fruit Bunch and Oil Palm Cake

Figure 3.B designates the growth performance of the diverse ratios of the Empty Fruit Bunch and Oil Palm Cake. The results depict 180:20 for the Empty Fruit Bunch (EFB) and Oil Palm Cake (OPC) as the best growth respectively for the different samples tested. The entire growth formulation of the substrate terms the substrate capacity to support the growth of the algae. The Positive control made with the two novel synthetic media had a much lower growth performance to the other ratios.

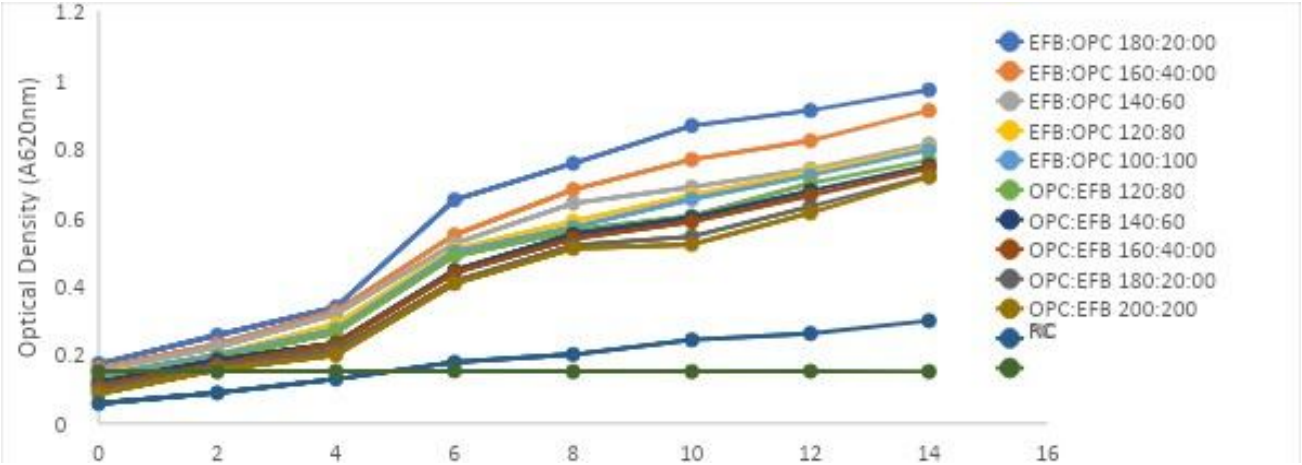

Fig 3.B: Growth selection of *Chlorella vulgaris* in different ratio of Empty Fruit Bunch and Oil Palm Cake

3.4: Optimization processes for *Chlorella vulgaris* growth

The following parameters were optimized for the growth of *Chlorella vulgaris*; temperature, salinity, pH, photoperiod, photoinhibition.

Figure 3.C describes the effect of different temperature variations on the optimal conditions for the biomass production. The results present the growth pattern of the 30ºC which had a lag phase between the first days. The negative control which was uninoculated extract. The positive control was novel synthetic medium inoculated with the 3-day old culture of the *Chlorella* had no significant increase. 20 ºC and 35ºC had noticeable growth on the medium, after the exponential increase of the cell, suggesting the presence of soluble nutrients to the algal cells at the temperature levels tested.

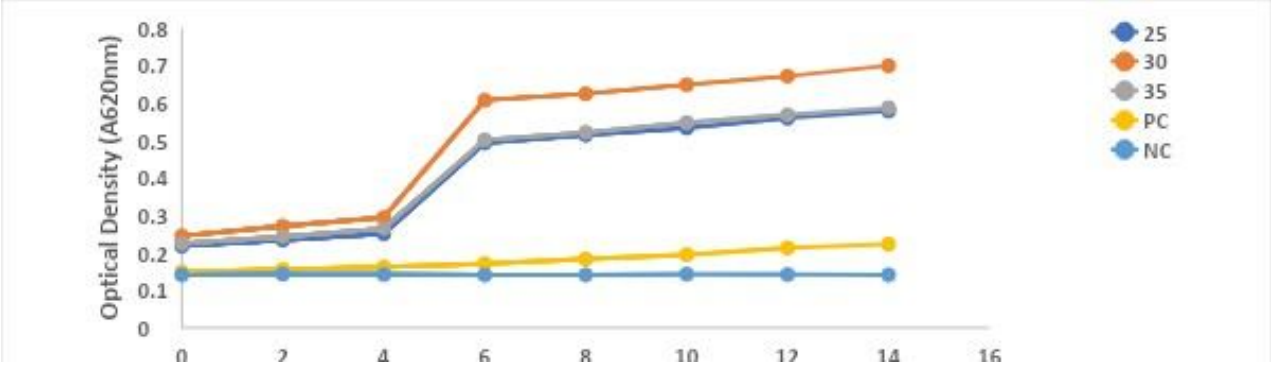

Fig 3.C: Effect of incubation temperature on *Chlorella* growth
Key: PC= Positive Control (Inoculated Novel Synthetic Medium)
NC= Negative Control (Uninoculated extract)

3.5: Effect of salinity on *Chlorella* growth

Figure 3.D shows the response of *Chlorella sp.* to saline environments. The superlative growth was observed for the 10 ppm of sodium chloride, the log phase started from the day 0 (0.228 abs) to day 2 (0.246 abs). Also, the 15ppm had a high biomass growth. The positive control had a lag phase on the first day and followed by an exponential phase. The number of cells created was limited by growth factor and as a result the rate of cell growth matches the cell death. The negative control remained unchanged for the entire growth period. Congruently other salinity intensities had different growth but not significant.

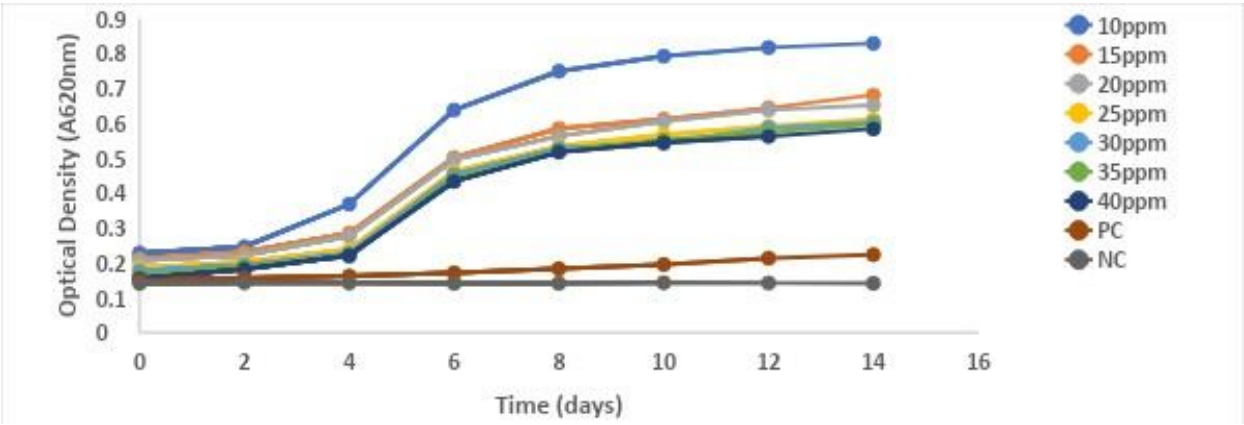

Fig 3.D: Effect of salinity on *Chlorella* growth
Key: PC= Positive Control (Inoculated Novel Synthetic Medium)
NC= Negative Control (Uninoculated extract)

3.6: Effect of different pH values on *Chlorella* growth using mixture of Empty Fruit Bunch and Oil Palm Cake Extract

Figure 3.E is the response of the biomass feedstock to fluctuating level of the pH ranges. The result recommends that the pH 6.0 had the best biomass growth and build up with the lag phase of between 0-3days. Biomass build up pH at 7 – 9 had no striking significant difference.

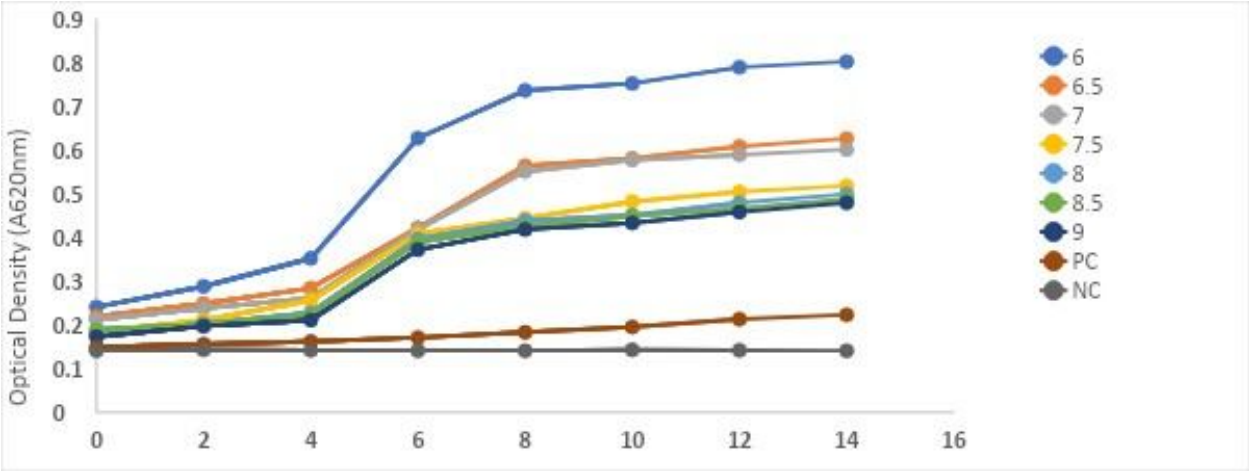

FIG 3.E: Effect of different pH values on *Chlorella* growth using mixture of Empty Fruit Bunch and Oil Palm Cake extract
KEY: PC= Positive Control (Inoculated Novel Synthetic Medium)
NC= Negative Control (Uninoculated extract)

3.7: Effect of photoperiod on *Chlorella* growth using Oil Palm Cake and Empty Fruit Bunch

Figure 3.F presents the growth pattern of the isolates after their previous exposure to the 12:12 and 6:18 photoperiod for the blend of the oil palm extract, the study suggests the later photoperiods had a significant response compared to the negative and the positive control. The study observed an increase in the population of the cells with a log/exponential phase between 0 - day 3. The positive control was observed to have corresponding rise in biomass but performed least.

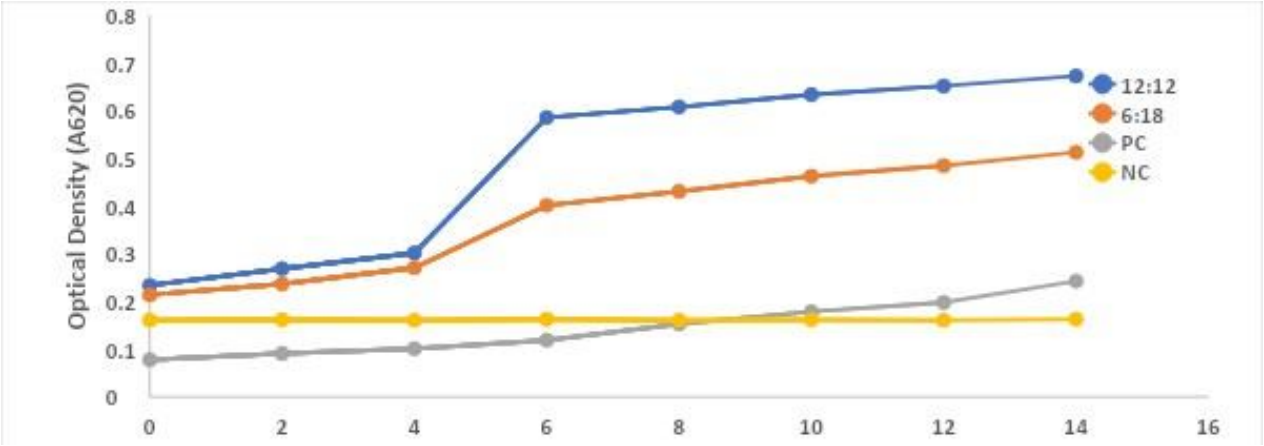

Fig 3.F: Response of *Chlorella* sp. to photoperiod
Key: PC= Positive Control (Inoculated Novel Synthetic Medium)
NC= Negative Control (Uninoculated extract)

3.8 Effect of photoinhibition to *Chlorella* growth

Figure 3.G shows the growth curve of the cultured chlorella cells under 12:12 and 6:18 periods. The result suggests there is no significant difference in the growth between the periods of exposure to light. The positive control was observed to have increased from day 2 to day 5.

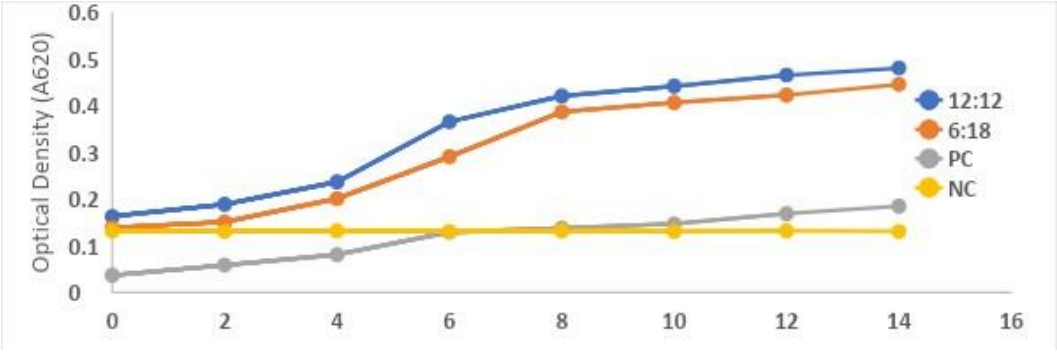

Fig 3.G: Effect of photoinhibition on the *Chlorella* sp.

KEY: PC= Positive Control (Inoculated Novel Synthetic Medium)

NC= Negative Control (Uninoculated extract)

4.0 Discussion

Agricultural industries produce an enormous quantity of agro industrial residue and there is a necessity to exploit these wastes to yield valuable products. Oil palm residues are one of the wastes spawned in large amount by oil palm mill processing industries and during the manufacturing of palm oil [17]. The Empty Fruit Bunch and Oil Palm Cake were both utilized as good source of nutrients for the growth of *Chlorella vulgaris*. Protein composition is vital for biosynthesis of active enzymes and co-enzymes, comparatively protein content is needed for functional system and supplementation [18]. The protein content for OPC, EFB and algae biomass is (1.30 ± 0.18, 0.80 ± 0.08 and 1.15 ± 0.06 ppm). Carbohydrate composition is a significant nutrition for algal growth [19]. OPC and EFB had carbohydrate level of 29.83 ± 0.29 and 29.29 ± 0.58 ppm which favored the growth of the microalgae. It has been pragmatic that about 80% of the entire cost of production in microalgae cultivation is carbon substrate [20]. These carbohydrates synthesized are amalgamated into the cell wall as a prospective feedstock for citric acid production. The grinded oil palm waste underwent aqueous extraction and was analyzed for their physicochemical properties. [21] recounted a huge amount of organic and inorganic nutrients tied to agro residue, these residues serve as reservoir of nutrients. pH of a medium affects bioavailability of nutrients. The pH of the oil palm waste was (OPC=5.15±0.02, EFB=5.36±0.06). [22] reported that extreme pH conditions may impede the growth of organisms. OPC and EFB were found to have high sulphate and nitrate content. Sulphate (ppm) (OPC=36.35±0.46, EFB=50.68±0.49) and Nitrate (ppm) (OPC=31.05±0.48, EFB=40.33±0.98). According to [23] these ions are essential to enhance the growth of *Chlorella* sp. [24] also reported that the ions have been noted to be compulsory for simulation of microalgal growth triggering blooming of the organisms. Optimal conditions are fundamental for cultivation of microalgae [25]. In this study, the optimal temperature recorded with the oil palm waste was 30^{o}C. The results of this study agree with the findings of [25]. who reported the same temperature. [26] reported that temperatures above 30^{o}C do not favour maximum growth activity in *Chlorella vulgaris*. Salinity is a quantity of ions and salts present in a medium for proper growth of microalga [27]. [28] reported that a negative trend in the growth rate of the microalga was observed when the NaCl concentration was elevated above 15ppm, which affected the growth of cells. In this study, salinity concentrations of 10ppm had highest biomass quality as seen in figure 3.E. The work of [25] suggested that an optimum salinity of 10ppm was recorded. The optimal pH was observed to be 6.0, this agrees with the reports of [29]. They reported that pH lenience of 7.0 is critical for the growth microalgae and a high throughput of biomass was observed. The determination of the photoperiod is a crucial procedure in the selection of media activity, for the growth of microalgae which states that more biomass was accumulated in the 12:12 period of growth cultivation than in 6:18 period. The result of this study agrees with the report of [30] which states that 12h day and light encouraged the production of biomass. But [31] investigations revealed that 24h dark cultivation conditions lower biomass production. After inoculation of the microalgae to oil palm extract in a ratio of 180:20 of EFB and OPC, at day 0 the cell density was almost zero because the cells have to adapt to the physiological conditions of the new environment. During the day three, the (lag phase) the microalgal showed slow growth as the cells allocate most resources to the physiological adaptation induced by the new environment [32]. The lag phase was proceeded by a rapid exponential phase which is characterized by cell doubling, doubling will continue at a constant rate so both the number of cells and the rate of population doubles each consecutive time period. As the nutrient becomes exhausted, the growth phase is decelerated and the cell biomass augmented linearly. Then the

growth phase remained stationed till lapse of incubation period because the nutrient has depleted and the biomass concentration decreased. Thus, these optimum conditions were observed to be very good for the algal growth.

5.0 CONCLUSION

Elaeis guineensis extract is a low-cost carbon substrate effective for the growth of Microalgae with high biomass production. The optimization revealed pH 6.0, temperature 30^{o}C, salinity 10ppm and photoperiod 12:12 day: night as optimal condition for the growth of *Chlorella vulgaris* biomass. The media formulations used in this study revealed clearly distinct phases viz., lag, exponential and stationary phases, which are characteristic of typical growth dynamic patterns according to the sigmoid curve (batch growth culture). Cultivation of microalgae on agro-waste can make an important contribution to transition to a more sustainable society.

Disclosure of conflict of interest

Authors have declared that no conflict of interest exist

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