Effect of pH on Oil Extraction from *Chlorella spp*.

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ABSTRACT

In this work, a brief introduction, and the meaning, historical background, chemical composition, and uses of microalgae is presented. Cultivation of *Chlorella spp*. was also achieved in a constructed three 2-litre bubble-column photobioreactor using bold basal medium at varying pH levels of 5.5, 6.0, and 6.5.

The bubble-column photobioreactor with pH of 6.5 provided better performance when compared to the other photobioreactors, with a maximum value of 0.425 for optical density and 3.60×10^{3} g/ml.day for growth rate. The bubble-column photobioreactor with pH of 6.0 had a maximum value of 0.420 and 3.23×10^{-3} g/ml.day for optical density and growth rate, respectively. While the bubble-column photobioreactor with pH of 5.5 had a maximum value of 0.410 for optical density and 3.19×10^{-3} g/ml.day for growth rate. The photobioreactors were operated at the same aeration rate and light intensity. In terms of oil yield, bubble-column photobioreactor with pH of 5.5, 6.0 and 6.5 gave yields of 30%, 44% and 54% respectively.

(Keywords: microalgae, bubble-column photobioreactor, Chlorella spp., oil extraction, optical density, specific growth rate)

INTRODUCTION

One of the most serious environmental problems today confronting humanity is global warming caused primarily by the heavy use of fossil fuels. The carbon dioxide (CO_2) generated by power plants and industry can be recovered with technology such as chemical absorption. Microalgae are potential candidates for using excessive amounts of CO_2, also their cultivation are capable of fixing CO_2 to produce energy and chemical compounds in the presence of sunlight.

Nigeria, as a developing nation depend on fossil fuels (coal, oil, and natural gas), which are finite and nonrenewable energy sources. Fossil fuels currently provide more than 90% of all energy consumed in Nigeria-Nearly three-quarter of the electricity, and virtually all of the transportation fuels [1]. Nigeria for example, is a monocultural economy, depending solely on petroleum for its fuel needs. More than 80% of the oil used in Nigeria today for the production of diesel is derived from non-renewable sources.

Nigeria exports about 2.4 million barrels of oil per day. At an average cost of $100 per barrel. The huge proceeds from this crude oil has not in any way been used for the betterment of the average Nigerian. This has given rise to massive poverty, economic depression, crime, youth restiveness, violence, clamor for resource control, agitation for secession, corruption, militancy, etc. Although the exact time at which fossil fuels will run out is debated, it is probably inevitable that fossil fuel supplies will decline in the future and will become even more expensive.

Currently, advanced countries of the world such as the United States and Europe who are the major importers of our crude resources are researching into renewable energy sources. As they secure a breakthrough, which will certainly happen, their demand for these resources will decline thereby jeopardizing our economy. Therefore, there is an urgent need for alternatives to fossil fuels, such as biofuels, including biodiesel, ethanol, and other types of biomass-derived fuels.

In order to end Nigeria's and other developing countries' addiction to fossil oil and combat global warming, we should begin to look to alternative oil and fuel sources. Among other edible and non- edible oil-bearing feedstocks, microalgae holds a great deal of potential in this regard. Compared with other terrestrial crops which take

a season to grow and only contain a maximum of about 5 percent dry weight of oil, microalgae grow quickly, does not compete with edible crops for food and contain high oil content [2]. Table 1 lists the potential yields of oil produced by various crops and compared with microalgae.

Table 1: Oil Yields Based on Crop Type.

Crop	Oil yield (gallons/acre)
Corn	18
Soybean	48
Canola	127
Jatropha	202
Coconut	287
Oil Palm	636
Microalgae	6283 - 14641

Source: [2]

The two basic microalgae cultivation systems are raceway pond and photobioreactors. Commercial microalgae productions are mainly performed in raceway ponds, with the principal advantage of using free light energy from the sun. However, large-scale open algae cultures results in low cell densities with expensive harvesting procedures and unfavorable economics in addition to difficulties with species control. These systems are easily contaminated by other organisms, so it is particularly difficult to maintain an axenic monoculture in raceway ponds. Thus they have only proven adequate for the cultures of specific species that grow in highly selective environments that exclude contaminating organisms.

Furthermore, settlings cause low yields unstable microalgae population and difficulty in distributing nutrients [3]. Based on these, photobioreactors have been developed as the economic alternative option for the cultivation of specialized microalgae stains for the production of specific biochemicals and oil. Compared to raceway ponds, photobioreactors have low risk of contamination, have significantly higher biomass and oil productivity or yield (in terms of quality and quantity), no loss of water by evaporation and the fact that system cleanability is possible, thus there is the ease of switching algae species.
Of all factors affecting microalgae oil extraction, pH is profound. Therefore the objective of this work is to determine the effect of pH on oil extraction from *chlorella spp*. using bubble-column photobioreactor.

Meaning and Classification of Microalgae

Algae are organisms with unicellular or simple multicellular body plan that are able to manufacture their own food material by photosynthesis. Algae are used in food industries and cosmetics. Also, algae have the ability to absorb metals in biotreatment [4].

Microalgae are prokaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure. Examples of prokaryotic microorganisms are cyanobacteria (cyanophyceae) and eukaryotic microalgae are for example green algae (Chlorophyta) and diatoms (Bacillariophyta) [5].

Found in both marine (salt water) and fresh water habitats, they measure no more than a few hundred micrometers (µm) in size [6, 7]. In spite of their miniscule size, microalgae play a pivotal role in the maintenance of life on Earth – they alone produce half of all the atmospheric oxygen on the planet. It is also not surprising to note that microalgae possess an incredible biodiversity; estimates vary between 200,000 and a few million different species, and only a tenth of this variety has been scientifically identified to-date. In contrast, the total number of plant species identified number just over 315,000 [7].

For the past 50 years, extensive research has been performed on microalgae and how they can be used in a wide variety of processes or to manufacture many practical and economically important products. The first large-scale culture of microalgae started in the early 1960s in Japan by Nihon Chlorella with the culture of *Chlorella* [8].

The interest in using microalgae for renewable energy began in the 1970s during the first oil crisis. The U.S National Renewable Energy Laboratory (NREL) through the Aquatic Species Program (ASP), launched a specific research and development (R&D) program dedicated to alternative renewable fuels, including biodiesel from microalgae that lasted from 1978 to 1996 [9].

From 1987 to 1990, an outdoor test facility of two 1000m^2 high-rate ponds was operated in Roswell, New Mexico. It was concluded that the use of microalgae for low cost production of biodiesel was technically feasible, but still needs considerable long term R&D to achieve the high productivities required.

Other objectives of this NREL R&D program was to improve algae strains by looking for genetic variability between algal isolates, attempting to use flow cytometry to screen for naturally occurring high oil individuals, and exploring algal viruses as potential genetic vectors. However, in 1995, the Department of Energy reduced the budget allocated to funding this program and it was discontinued before these experiments could be carried out beyond the preliminary stages [9].

The recent price volatility of crude oil and the expected future price increase, tied with the urge to reduce pollutant emissions and greenhouse gases, have created a new interest in the production of biodiesel using microalgae. For example, several companies were created or have entered this market niche, selling either entire processes or key process units, such as photobioreactors with optimized designs to cultivate microalgae for biodiesel production and other applications [10].

Nowadays, microalgae are seen as an alternative feedstock for biodiesel production, being the target of a large number of consortiums, private and public organizations investment in R&D, aiming to use the most effective and cheap technology to produce large amounts of oil. They are considered to be a second generation feedstock together with other biomass sources, such as Jatropha, lignocellulosic materials, agricultural residues, and systematically grown energy crops, with high potential yield of biofuels and that are not used as food source for human consumption [11].

Microalgae are capable of fixing CO_2 from the atmosphere and water to produce biomass more efficiently and rapidly than terrestrial plants. Numerous algal strains have been shown in the laboratory to produce more than 50% of their biomass as oil with much of thisastriacylglycerides (TAGs), also called triglycerides, the anticipated starting material for biodiesel fuels [2].

Most of the observations of high oil content come from algal cultures grown under nutrient (especially nitrogen, phosphorous, or silicon) limitation. Oil content varies in both quantity and quality with varied growth conditions. The possibility that microalgae could generate considerably more oil than typical oil seed crops is an exciting opportunity.

Microalgae grow extremely rapidly and many algae species are rich in oil. For instance, heterotrophic growth of *Chlorella protothecoides* can accumulate oil as high as 55% of the cell dry weight after 144 hours of cultivation with feeding of corn powder hydrolysate in fermenters [12]. Oil levels of 20-50% are common in microalgae [2]. The whole technical process in the production of biodiesel from microalgae has been well investigated in recent years. An additional benefit of growing algae as a biofuels feedstock is that they can be cultivated in non-productive (i.e., non-arable) land that is unsuitable for agriculture or in brackish, saline, and waste water that has little competing demand, offering the prospect of a biofuel that does not put pressure on agricultural land.

Chemical Composition of Microalgae

Algae are made up of eukaryotic cells (Figure 1). These are cells with nuclei and organelles. All algae have plastids, the bodies with chlorophyll that carry out photosynthesis. But the various lines of algae have different combinations of chlorophyll molecules. Some have only Chlorophyll A, some A and B, while other lines, A and C.

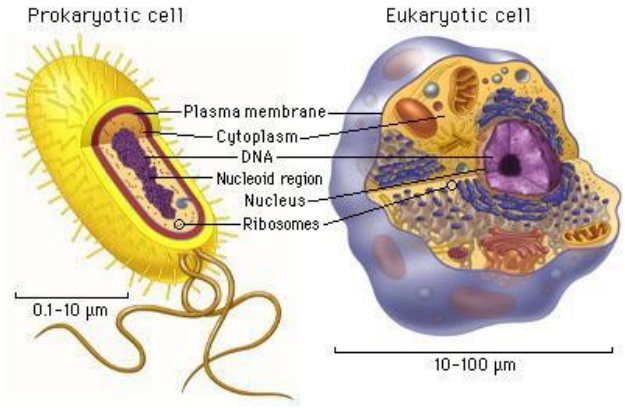

Figure 1: Scheme of Prokaryotic and Eukaryotic Cells. (Source: 13).

All algae primarily comprise of the following in varying proportions: Proteins, Carbohydrates, Fats and Nucleic Acids. While the percentages vary with the type of algae, there are algae types that comprised up to 40% of their overall mass by fatty acids. It is this fatty acid (oil) that can be extracted and converted into biodiesel.

Uses of Microalgae Carbon Dioxide Sequestration, Mitigation, and Capture

Microalgae is envisioned to have a great potential to diminish or capture carbon dioxide (CO_2) in the atmosphere and to provide a very promising alternative to current greenhouse gas emissions mitigation strategies. Two main CO_2 mitigation strategies are normally used [14]: the chemical reaction-based approaches and the biological mitigation.

On one hand, the chemical reaction-based CO_2 mitigation approaches are energy-consuming, use costly processes, and have disposal problems because both the captured carbon dioxide and the wasted absorbents need to be disposed of. On other hand, the biological CO_2 mitigation has attracted much attention in the last years since it leads to the production of biomass energy in the process of CO_2 fixation through photosynthesis [15].

The laboratory studies carried out by Zeiler et al. [16] using a green algae Monoruphidiumminutum, demonstrated that this algae can efficiently utilize simulated gases containing high levels of carbon dioxide, as well as sulfur and nitrogen oxides, as a feedstock to produce substantial biomass for oil extraction and other uses. The green algae Chlorophyta showed the ability to fix CO_2 while capturing solar energy with an efficiency of 10–50 times greater than that of terrestrial plants [14].

Flue Gas Reduction and Waste Water Treatment

Microalgae also help in the removal of flue gasses from steel industries and thermal power plant. Growing microalgae cells get essential nutrition (though harmful to humans) from wastewater in which they grow. Extensive studies have been carried out by using wastewater for the cultivation of algae biomass, especially for the removal of nitrogen and phosphorus salts from effluents [17, 18, 19], which would otherwise result in eutrophication if dumped into lakes and river. In addition to nitrogen and phosphorus removal, other studies have also been conducted for the removal of heavy metals from wastewater [20, 21].

Microalgae cells can effectively grow in both fresh and saline water having salt concentrations up to twice that of seawater. *Dunaliella salina* can grow in salinity levels higher than 200 g NaCl L^{-1} and *Spirulina platensis* can grow in highly alkaline medium with a pH up to 10 [22]. Water is usually occurring in treated or untreated livestock wastewater and they can be reprocessed to conserve resources as well as to reduce the culture medium cost. Conclusively, microalgae need not compete with other users for fresh water and cultivating algae biomass by utilizing wastewater can be considered as biological remediation phenomenon under wastewater management.

Production of Oil for Biodiesel

In addition to other uses, the best possible use of microalgae oil is certainly its transformation to a biofuel, especially biodiesel. Microalgae can be used for oil extraction and subsequently biodiesel production. Biodiesel is defined as a fuel comprised of mono-alkyl esters of long chain fatty acids derived from triacylglycerol (TAG). This can be produced from renewable resources such as animal fat, vegetable oil or oil plants as the first and second generation feedstock like soybean, sunflower, Jatropha, palm oil [23].

Nowadays, there has been an increasing interest in looking for new oil feedstock for biodiesel production especially non-food feedstock to avoid the food-fuel conflict. Microalgae are seen as non-food feedstock promising candidates for the industrial production of biodiesel because of their advantages of higher photosynthetic efficiency, higher biomass production and faster growth compared to other energy crops. In fact, microalgae have the highest oil yield among various plant oils, and the oil content of some microalgae has up to 80% and the compositions of microalgae oils are mainly TAG which, in terms of quality, is the right kind of oil for producing biodiesel.

Production of Bioactive Compounds for Medical, Pharmaceutical and Industrial Uses

Depending on the microalgae species various high-value chemical compounds may be extracted such as pigments, antioxidants, b-carotenes, polysaccharides, triglycerides, fatty acids, vitamins, and biomass, which are largely used as bulk commodities in different industrial sectors (e.g., pharmaceuticals, cosmetics, nutraceuticals, functional foods, biofuels). Also, algae hydrocolloids alginate, agar, and carrageenan are produced from seaweeds (especially macroalgae) and largely used as viscosity modifying agents in foods and pharmaceuticals [24].

Because the production of these fine chemicals and bioactive compounds normally demands the use of monocultures and controlled cultivation systems for a highest productivity and production efficiency, this has led to the development of large-scale photobioreactors.

Microalgae have been found to contain several different types of sterols, including clionasterol isolated from Spirulina sp. that has been shown to increase the production of plaminogen-activating factor in vascular endothelial cells and thus facilitate cardiovascular disease prevention [24]. Additionally, several antioxidant compounds (e.g., dimethylsulfoniopropionate, mycosporines or mycosporinelike amino acids, b-carotene, astaxanthin and other carotenoids) have been isolated from microalgae sources, having the potential to protect against oxidative stress, cause of a wide spectrum of diseases and ageing.

Muriellopsis sp. among other microalgae is able to accumulate high levels of carotenoids such as lutein (normally presented in dark, leafy green vegetables, such as spinach and kale, as well as in corn, egg yolk, and some other foods with yellow color) that is used for the prevention and treatment of degenerative diseases [25].

Food and Feeds for Humans, Aquaculture, and Farm Animals

Consumers are progressively more concerned about debilitating illnesses (e.g. cholesterol, heart disease, osteoporosis, and cancer) and are not just relying on prescription medications to treat or prevent these illnesses, but increasingly examining the link between their diet and health. Traditionally nutritional supplements derived from plants have been used and predominated in the market. Nevertheless, the health benefits of aquatic microorganisms such as microalgae are being investigated and more recognized and appreciated, within the last three to four decades, especially since the introduction of probiotic supplements [24]. Microalgae is viewed as having a protein quality value greater than other vegetable sources, for example, wheat, rice, and legumes, but poorer than animal sources, for example, milk and meat [26].

Spirulina (Arthrospira) and Chlorella (a unicellular green microalga) are both considered edible algae that have been widely studied both in terms of their component molecules and their biological activities [15]. Considerable efforts have been made to promote the microalgae use in human food, aquaculture and animal feed. However, high production costs and fear of toxicological contamination have limited algae application to expensive *healthy* foods. So far, microalgae culture have been more successful for food source and feed additive in the commercial rearing of many aquatic animals (e.g., rearing larvae and juveniles of many commercially important mollusks, penaeid prawn larvae, crustaceans and fish) both freshwater and marine.

S. platensis and Spirulina maxima are the most popular, in terms of human consumption. Spirulina sp. is currently largely cultivated for use as a healthy food, since it boosts the immune system, helping to prevent both viral infection and cancer. It has also been reported to increase the number of lactic acid bacteria in the gastrointestinal tract as a result of a dietary supplement for promoting healthy hormonal balance in adult human. Additionally, it has a high nutritional value due to its protein content of 55–70% of total dry weight. For this reason Spirulina have been used as a food source in Asia for more than 1,000 years and in Lake Texcoco (Mexico), during the Aztec civilization, some 700 years ago (e.g., dried and sold as small cakes to incorporate in bread) [24].

Many species of microalgae have been shown to contain a high percentage of oil [2]. Research in biotechnology has proven that when microalgae oil is extracted it can further be converted to biodiesel, biomethane, bioethanol, and biohydrogen [27]. Also, photobioreactors are better systems for culturing microalgae when

compared to raceway ponds. Microalgae have displayed the potential to curb emerging environmental problems such as greenhouse effect and industrial water pollution. Less area is required by bubble-column photobioreactors when compared to conventional energy crops.

It is necessary to manipulate microalgae growth and cultivation environment in other to optimize oil and biomass productivity. A number of factors have been shown to influence the oil content of microalgae, such as nitrogen deficiency [28, 29]), phosphate limitation [29]), salt stress [30], and temperature fluctuation [31]. Light intensity and iron content of the medium also affect microalgae growth along with oil content [28].

Several studies have shown that oil tends to accumulate in nitrogen deficient conditions [32, 29]). In general, there is an inverse relationship between oil yield and nitrate concentration [29]. Currently, there is little information in literature on the effect of pH on oil yield and performance of microalgae strains (e.g., *Chlorella spp*.). Therefore the broad objective of the present study was to determine the effect of pH on microalgae oil extraction. Other specific objectives are to culture *Chlorella spp*. in a bubble-column photobioreactor and to determine the optical density, growth rate, specific growth rate and oil yield of *Chlorella spp*. cultured in the bubble-column photobioreactor.

MATERIALS AND METHOD

Collection and Identification of Sample

An axenic culture of *Chlorella spp.* was collected from the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. To confirm the identity of the plant, the collected material was viewed under x40 objective lens with a light microscope and identified using a dichotomous key [33]. The organisms were unicellular. The cells were seen as spherical, non-flagellate and unconstricted.

Sterilization of Materials

Three (3) glass jars of 2000ml capacity were properly washed with detergent in lukewarm water. They were then dried in a hot air oven at a temperature of 105^{o}C for 8 hours. Beakers, syringes and measuring cylinders were also sterilized by autoclaving (Figure 2) at 121^{o}C for 15 minutes. 70 % alcohol was also used to swab the work table area to prevent contamination.

Figure 2: An Autoclave used for Sterilization.

Preparation of Medium and Inoculation of Sub-culture

The basal medium reported by Endo et al. [34] and modified by Ogbonna *et al*. [35] was used to culture the *Chlorella spp.* biomass. The compositions are as stated in Tables 2 and 3.

Table 2: Composition of Modified Bold Basal Medium (BBM).

Salts	Amount (g/l)
Urea	1.2
KH_2PO_4	0.3
$MgSO_4.7H_2O$	0.3
$CaCl_2$	0.02
$NaNO_3$	0.05

Source: [35]

One liter volume of the basal medium was prepared in a volumetric flask by dissolving the salts (excluding the urea) in distilled water. The A5-solution, composed of the salts shown in Table 2 was prepared in another 1 L volumetric flask. An iron solution (Fe) composed of 25 g of $FeSO_4.7H_2O$ and 33.5 g of EDTA was also prepared in 1 L of distilled water. The medium used for culture was prepared by adding 0.16 ml of Fe-solution and 0.8ml of A5-solution to the basal medium mixture.

Table 3: Components of A5-Solution.

Salts	Amount (g/l)
H_3BO_3	2.86
$ZnSO_4.7H_20$	0.22
$MnCl_2.4H_20$	1.81
$CuSO_4.7H_20$	0.08
MoO_4	0.015

(Source: Ogbonna et al., 1997) [35]

The pH of the medium was adjusted to 6.5 before autoclaving at 121°C for 15 minutes. Filter-sterilized urea was added to the autoclaved medium after cooling to room temperature [35]. The inoculum collected was placed in the medium in the ratio of 4:1. 400 ml of the medium was inoculated with 100ml of the inoculum in sterilized glass jars using sterilized syringes. The glass jars were covered by plugging with cotton wool and then taken to a screen house and arranged on a table-top as shown in Figure 3. The culture was shaken three (3) times daily to prevent photo-inhibition.

Figure 3: Sub-Culture Arranged in the Screen House.

Components of the Bubble-Column Photobioreactor

A bubble-column photobioreactor was used for the culture. It is an assembly of a cylindrical column, a sparger, a system used to mix the culture (in most designs an aquarium air pump is used), and a lighting system. Three (3) cylinders of 2000ml capacity were used as the cylindrical column.

The cylinders are transparent to enable the transfer of light to the cells. The sparger provides aeration through small orifices and is the most practical way of creating mixing, enabling mass and nutrient transfer and providing CO_2 to the cells. A sparger was improvised using an air stone. Three (3) VENUSAQUA Aquarium Air Pumps with a maximum outlet of 3L/min and a power of 2.5W were used to achieve mixing. The air pump was connected to an electric socket. The outlet of the air pump was connected to the air stone using a tube. Light was supplied to the system using three (3) NOMI energy saver bulbs with a voltage of 150V and a power of 15W. The bulbs were placed at a distance of 60cm from the cylindrical column.

The cylindrical column was covered with foam sheets. A hole was made on the foam sheet through which the tube conveying air passes. The experimental set-up of the bubble-column photobioreactors is shown in Figure 4. The pH of the media in photobioreactors A, B and C were adjusted to 6.5, 6.0 and 5.5 respectively. The cultures were illuminated and sparged for eight (8) hours daily. 3ml of the culture was collected from each photobioreactor and their absorbance were determined using a spectrophotometer at a wavelength of 680nm. This was repeated every two days. The culture was maintained for 30 days.

Figure 4: Experimental Set-up of the Bubble-Column Photobioreactors.

Determination of Optical Density (OD), Growth Rate, Specific Growth Rate, and Oil Yield

The optical density was determined using aUV/visible 722*s* spectrophotometer (B. Bran Scientific & Instrument Company, England) at a wavelength of 680nm. 3ml of *Chlorella spp*. was collected from each culture using a sterilized syringe and placed in a cuvette. Sterilized distilled water was used as blank. Cell growth

rate (g/ml.day) is usually expressed as an increase in cell concentration over a given period of time. Growth rate can be determined using Equation 1.

$$\frac{dX}{dt} = \frac{X_2 - X_1}{t_2 - t_1} \quad [36] \qquad \textbf{(1)}$$

Since dry cell weight is the most accurate unit for cell concentration [36], the values for X_1 and X_2 were interpolated from the standard curve (Figure 4) while t_1 and t_2 are the days of cultivation.

The specific growth rate is the growth rate per unit cell. It is the cell growth rate divided by the cell concentration. Specific growth rate was calculated using Equation 2.

$$\mu = \frac{X_2 - X_1}{t_2 - t_1} \times \frac{1}{\chi} \quad [36] \qquad \textbf{(2)}$$

The oil yield (%) is calculated using Equation (3).

$$Y = \frac{Weight\ of\ Oil}{Weight\ of\ Sample} \times 100 \qquad \textbf{(3)}$$

Where, Y is oil yield (%)

Biomass Harvesting and Oil Extraction

The *Chlorella spp.* biomass was harvested by the method of centrifugation. Cultures A, B, and C were centrifuged to a volume of 98ml, 100ml, and 102ml ,respectively. The centrifuge was operated at 3000rpm for ten (10) minutes. The supernatant was discarded and the residue collected in a beaker. Using Bligh and Dyer [37] method modified by Onwuka [38]. Oil was then extracted from the wet residue.

RESULTS AND DISCUSSION

Results Used to Prepare Standard Curve

The results used in the preparation of the standard curve include the initial weight of the filter papers alone after drying, the absorbance of the different dilutions and the weight of the filter papers with residue. The results are presented in Tables 4 and 5.

Table 4: Dry Weights of the Different Dilutions of *Chlorella spp*.

S/N	Initial weight of filter papers (g)	Weight of filter papers with residue (g)	Dry weight of residue (*Chlorella spp.*) (g)
1	0.884	1.133	0.249
2	0.888	1.089	0.201
3	0.889	1.039	0.150
4	0.887	0.985	0.098
5	0.886	0.933	0.047
Control	0.888	0.888	-

Table 5: Absorbance of the Different Dilutions.

S/N	Absorbance (at 680nm wavelength)
1	2.501
2	1.760
3	1.580
4	0.960
5	0.520
Control	-

It can be seen from Table 3 that the dry weight of *Chlorella spp*. was decreasing progressively as the organism was diluted with distilled water.

From Table 4, it can be seen that the absorbance also decreases as the concentration of *Chlorella spp*. was diluted. When a sample is placed inside a spectrophotometer, a lamp provides a source of light. When light of a specific wavelength (in this case 680nm) passes through the sample, some are absorbed by the sample while others are passes through the sample. A denser sample will absorb more light while a less dense sample will absorb lesser light.

From Figure 5, the test tube with 10ml of *Chlorella spp*. has the highest absorbance (2.369) while the test tube with 6ml of *Chlorella spp*. + 4ml of distilled water has the least absorbance (0.501).

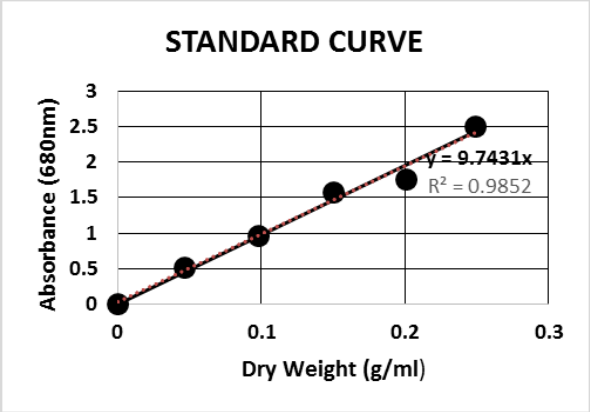

Figure 5: Standard Curve for *Chlorella spp.*

Results for Optical Density

Optical density readings determined using spectrophotometer are presented in Table 6.

Table 6: Optical Density of the Different Cultures.

Days of cultivation	Culture A (pH = 6.5)	Culture B (pH = 6.0)	Culture C (pH = 5.5)
2nd day	0.212	0.168	0.169
4th day	0.220	0.155	0.140
6th day	0.229	0.175	0.201
8th day	0.275	0.221	0.224
10th day	0.301	0.270	0.244
12th day	0.324	0.268	0.263
14th day	0.394	0.331	0.325
16th day	0.420	0.357	0.360
18th day	0.424	0.365	0.387
20th day	0.425	0.396	0.392
22nd day	0.441	0.398	0.410
24th day	0.420	0.420	0.405
26th day	0.415	0.378	0.397
28th day	0.399	0.372	0.382
30th day	0.394	0.365	0.351

As can be seen in Figures 6, 7, and 8, in the first 2-5 days of cultivation, *Chlorella spp*. experienced a lag phase of growth. The lag phase is a period which the cells use to adapt to the medium. The length of the lag phase depends on many factors such as the cell strain and the difference between the conditions of the sub-culture and the main culture.

From the 8th – 14th day of cultivation, the cells experienced a log phase. The log phase is characterized by a constant growth rate. After the adaptation phase, the cells starts to grow and the growth rate continues to increase gradually until it reaches a maximum constant value. This phase is also called the exponential growth phase because the cell number increases exponentially with time. Subsequently, the organism underwent a stationary growth phase. In this phase, there is no net increase in cell concentration.

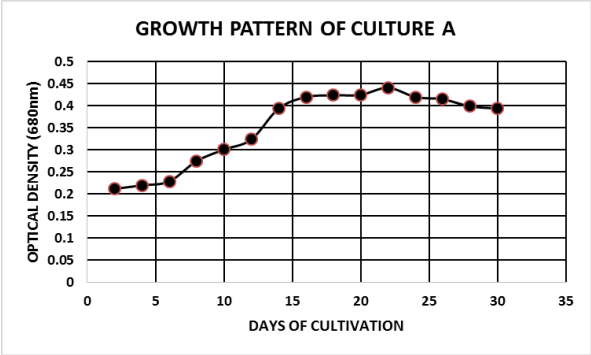

Figure 6: Growth Pattern of Culture A,

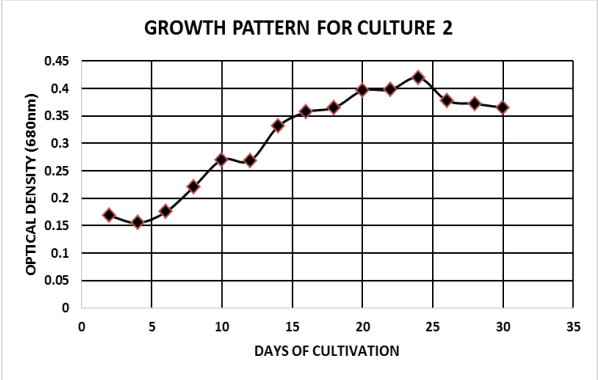

Figure 7: Growth Pattern of Culture B.

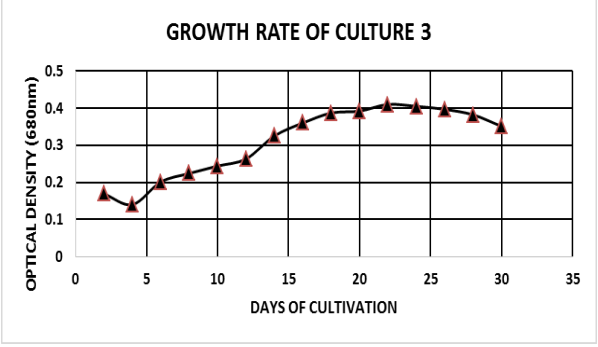

Figure 8: Growth Pattern of Culture C.

As time advances, the growth rate starts to decrease while the death rate continues to increase until there is no net increase in the cell concentration. In other words the growth rate is equal to the death rate. This happens due to depletion of nutrients and formation of harmful secondary metabolites.

From the 24^{th} - 30^{th} day of cultivation, the cell concentration starts to decline because the death rate is higher than the growth rate. However, maximum biomass productivity was observed in culture A while the minimum biomass productivity was observed in culture C. Figures 6, 7 and 8 are similar to that of FAO [39].

Results of Growth Rate, Specific Growth Rate and Oil Yield

Using Equations 1 and 2 the growth rate and specific growth rate for each day was calculated. The values for growth rate and specific growth rate for the three (3) cultures are presented in Tables 7 and 8, respectively.

Table 7: Growth Rate of the Different Cultures.

Day of cultivation	Growth rate for Culture A (g/ml.day)	Growth rate for Culture B (g/ml.day)	Growth rate for culture C (g/ml.day)
2^{nd} – 4^{th} day	4.10×10^{-4}	-6.65×10^{-4}	-1.49×10^{-3}
4^{th} – 6^{th} day	4.60×10^{-4}	1.03×10^{-3}	3.13×10^{-3}
6^{th} – 8^{th} day	2.37×10^{-3}	2.36×10^{-3}	1.18×10^{-3}
8^{th} – 10^{th} day	1.33×10^{-3}	2.52×10^{-3}	1.03×10^{-3}
10^{th} – 12^{th} day	1.18×10^{-3}	-1.00×10^{-4}	9.75×10^{-4}
12^{th} – 14^{th} day	3.60×10^{-3}	3.23×10^{-3}	3.19×10^{-3}
14^{th} – 16^{th} day	1.34×10^{-3}	1.34×10^{-3}	1.80×10^{-3}
16^{th} – 18^{th} day	2.05×10^{-4}	4.10×10^{-4}	1.39×10^{-3}
18^{th} – 20^{th} day	5.00×10^{-5}	1.59×10^{-3}	2.55×10^{-4}
20^{th} – 22^{nd} day	8.20×10^{-4}	1.05×10^{-4}	9.25×10^{-4}
22^{nd} – 24^{th} day	-1.08×10^{-3}	1.13×10^{-3}	-2.55×10^{-4}
24^{th} – 26^{th} day	-2.60×10^{-4}	-2.16×10^{-3}	-4.10×10^{-4}
26^{th} – 28^{th} day	-8.20×10^{-4}	-3.10×10^{-4}	-7.70×10^{-4}
28^{th} – 30^{th} day	-2.55×10^{-4}	-3.60×10^{-4}	-1.59×10^{-3}

Table 8: Specific Growth Rate of the Different Cultures.

Day of cultivation	Specific growth rate for Culture A (day^{-1})	Specific growth rate for Culture B (day^{-1})	Specific growth rate for culture C (day^{-1})
2^{nd} – 4^{th} day	0.0185	-0.0401	-0.0939
4^{th} – 6^{th} day	0.0199	0.0608	0.1789
6^{th} – 8^{th} day	0.0916	0.1161	0.0541
8^{th} – 10^{th} day	0.0450	0.1000	0.0429
10^{th} – 12^{th} day	0.0368	-0.0036	0.0375
12^{th} – 14^{th} day	0.0977	0.1051	0.1057
14^{th} – 16^{th} day	0.0321	0.0380	0.0509
16^{th} – 18^{th} day	0.0047	0.0111	0.0363
18^{th} – 20^{th} day	0.0011	0.0407	0.0064
20^{th} – 22^{nd} day	0.0185	0.0026	0.0225
22^{nd} – 24^{th} day	-0.0244	0.0269	-0.0085
24^{th} – 26^{th} day	-0.0061	-0.0527	-0.0099
26^{th} – 28^{th} day	-0.0196	-0.0081	-0.0193
28^{th} – 30^{th} day	-0.0063	-0.0095	-0.0423

Using Equation 3 for calculating the oil yield, culture A with a pH of 6.5 produced the highest quantity of oil (54%) while culture C with a pH of 5.5 produced the lowest quantity of oil (30%). The oil yield for culture B was 44% with pH of 6.0.

CONCLUSIONS

The recent hype in microalgae biotechnology is due to its high oil content and its potential as a biodiesel feedstock. Vertical reactors such as bubble-column photobioreactors have proven to be a reliable system for culturing microalgae. pH has effect on the optical density, growth rate, specific growth rate and oil yield of *Chlorella spp*. cultured in a photobioreactor.

This study revealed that at the different pH levels *Chlorella spp*. was cultured, the optical density, growth rate and specific growth rate increased, remained stationary for a while and decreased as time progressed. Oil was produced from each culture irrespective of pH. Microalgae cultured at a pH of 6.5 produced the highest quantity of oil (54%) while microalgae cultured at a pH of 5.5 produced the lowest quantity of oil (30%). Also, among the pH levels studied, pH of 6.5 is the optimum pH for maximum oil production. Therefore, it can be concluded that pH has an effect on oil extraction of *Chlorella spp*.

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