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Growth of *Chlorella* sp. on Flue Gas

H. A. Neboh[1], O. K. Agwa[1] and G. O. Abu[1*]

[1]Department of Microbiology, University of Port Harcourt, P.M.B. 5323, Choba, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author HAN designed the study and wrote the first manuscript. Authors OKA and GOA managed the analysis of the study and reviewed the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To use the ubiquitous dirt scavenger, *Chlorella* sp. as an affordable, low tech transducing device in the interplay between energy utilization and extreme climatic and ecological impacts which is a key to sustainable development.
Methodology: *Chlorella* sp. was isolated from effluent samples of a fertilizer company in the Niger Delta. The *Chlorella* sp. was cultured using a mixture of effluent and river water and supplied with 2%, 5%, 10% and 15% flue gas from a 3.0 kW TEC gasoline powered generator. The cultures, in triplicate, were aerated with an aquarium pump and the growth conditions were monitored as cell density (OD 600nm), cell number (cells/ml), lipid (mg/g), dry matter (mg/l) and CO_2 utilization (g/l) for 14 days at an ambient temperature of 29±2ºC.
Results: The flue gas solution supported the growth of the *Chlorella* sp. with no lag phase. The CO_2 removal in the culture was quantitatively measured as 0.0428g/l, 0.1214g/l, 0.2094g/l and 0.3219g/l for the 2%, 5%, 10% and 15% flue gas concentrations respectively. The highest increase in cell number was recorded with the 15% flue gas concentration. Dry cell weight of 3.30mg/ml, 4.40mg/ml, 5.40mg/ml and 5.60mg/ml were obtained for the 2%, 5% 10% and 15% flue gas concentrations respectively. Lipid yields of 45mg/g, 47.5mg/g, 52.5mg/g, and 57.5mg/g of cell dry weight were obtained on the 14th day for the respective flue gas concentrations.
Conclusion: The results demonstrate that an inexpensive growth medium can be realized from the flue gas, the effluent and river water for cultivation of the *Chlorella* sp.

**Corresponding author: E-mail: gideonabu1@yahoo.com, hopenwanagu@yahoo.com;*

for biomass and lipids. The biomass can be used as food supplements (nutriceuticals) and biochemicals while the lipid can be converted into biofuel as biodiesel. The flue gas is sequestered reducing environmental pollution (a clean development methodology) which could mitigate the effects of global warming in an environmentally friendly way.

Keywords: Chlorella sp.; flue gas; pollution; biomass; biodiesel.

1. INTRODUCTION

The search for alternate forms of energy is becoming more intense with the depletion of fossil fuels and accumulation of flue gases that make current practices and conditions unsustainable [1]. While there is still much debate on the effects of increased CO_2 levels on global climate, many scientists agree that the projected increase could have a profound effect on the climate. Global warming has been one of the most serious environmental problems, Dianursanti and Annondho [2], attributed its main cause to increasing flue gases like CO_2, NOx, SOx and trace metals in the atmosphere which result from the combustion of fossil fuels for energy production [3]. In recent years, many attempts have been made to reduce the quantity of these flue gases in the atmosphere. According to Villarejo et al. [4], *Chlorella* sp. has been considered a widely used unicellular model in ecotoxicological research, photosynthesis regulation and CO_2 fixation due to its high valued potential substances such as carotene, protein including 19 amino-acids, vitamins and minerals especially beta-carotene and vitamin B12, highest known source of nucleic acid (RNA/DNA) and highest known source of chlorophyll (5-10 times) greater than any other plant, also rich in unsaturated fatty acids (around 80% of its total fatty acid content) [5], and due to their tolerance levels for high concentration of CO_2 and high efficiency in utilizing CO_2 during photosynthesis [6]. *Chlorella* is a genus of single cell green algae, belonging to the phylum chlorophyta; class chlorophyceae; order chlorellales; family chlorellaceae [7]. The name *Chlorella* was taken from the Greek word *chloros*, meaning green and the Latin diminutives suffix *ella*, meaning small [8]. Its cells are solitary, very small (2.0 - 10µm) and spherical, globular or ellipsoidal in shape. *Chlorella* sp. is among the fastest growing photosynthetic organism having carbon fixation rates an order of magnitude higher than those of land plants. It utilizes CO_2 as one of its main building blocks and is a viable option for anthropogenic CO_2 capture and sequestration [9]. This microalga can produce a variety of high value compounds that can be used to generate revenues, such as biofuels. Biofuels are considered as best alternate fuels because they are made from non-toxic and biodegradable resources [10]. They can utilize CO_2 from various sources and produce less SOx¸ NOx, and particulate emissions when burned. They contribute to sustainable development by reducing these gaseous emissions and thus mitigate the effects of climate change and global warming, [10]. Biodiesel can be produced from conventional crops and other sources such as vegetable oil, used frying oils, animal fats, soap socks and greases; these are the “First generation” biofuel. The “Second generation” biofuel oil produced from microalgae is becoming a promising supplemental oil source[11]; and the use of *Chlorella* sp. for the Second generation biofuel has proven to be more advantageous and promising than the aforementioned sources for the following reasons; The cultivation of *Chlorella* sp. does not need much land compared to that of terraneous plants[10]; due to its simple cellular structure *Chlorella* sp., has higher rates of biomass and grow extremely rapidly and is richer in oils than conventional crops [12]; Since vegetable oils may also be used for human consumption, biodiesel produced from vegetable oils can lead to an increase in the food-grade oil price, causing the cost of biodiesel to increase and preventing its usage; It can be harvested batch - wise nearly all year round providing a reliable and continuous supply of oil

[13];. The production of biofuels from *Chlorella* sp. can be coupled with flue gas mitigation, waste water treatment, production of high valued chemicals, many kinds of lipids, hydrocarbons and other complex oils [14]. The process has minimal negative impacts on the environment and it is sustainable.

Since the discovery of the importance of *Chlorella,* its cultivation has been of interest to many scientists. This has led to the development of various ways of culturing this organism. At present *Chlorella* biomass production has been achieved by; Photoautotrophic cultivation - which involves the use of solar energy and inorganic carbon (CO_2); Heterotrophic cultivation - which involves the microalga using organic carbon source [15]; Mixotrophic cultivation - in which they can undergo photosynthesis and use both organic compounds and inorganic carbon (CO_2) as a carbon source for growth. *Chlorella* sp. assimilates organic compounds and CO_2 as a carbon source, and the CO_2 released via respiration would be trapped and re-used under phototrophic cultivation [16]. We experimented the green technology of growing *Chlorella* sp. using flue gas emissions and waste effluent from a fertilizer company and water from a local river; to establish an inexpensive growth medium which also serves as a means of reducing the negative effects of gaseous emissions.

2. MATERIALS AND METHODS

2.1 Sample Collection

Water samples containing the microalga were aseptically collected into clean (2 litres) sampling jars from the outfall sampling point of Notore chemical company (fertilizer company) Onne, Rivers state and also from the River water (top 20cm) of the New Calabar River, Choba also in Rivers state. The water samples were transported to the laboratory in ice jackets. The samples were microscopically viewed under the microscope.

2.2 Assay of Physicochemical Properties

The physicochemical properties of the water samples were measured using standard analytical procedure [17]. The water samples were analyzed for the following physicochemical properties: Total Nitrogen, Chloride, Nitrate, Phosphate, Sulphate, Ammonia, Total Organic Carbon (TOC), Total Dissolved Solids (TDS), Conductivity, Iron, and Zinc.

2.3 Media Formulation and Growth Conditions

The water samples were filtered using the whatman's filter paper (no 1). The growth medium was formulated using effluent from Notore chemical company, Onne, Rivers state and river water from the New Calabar River, Choba. A synthetic medium was also used, it contained the following salts in g/l; potassium nitrate (0.132), silicate (0.066), monosodium phosphate (0.066), EDTA (0.066) and water (1000ml), at a pH of 7.5, it was autoclaved and allowed to cool. The synthetic medium was used in conjunction with the effluent and river water to induce blooming of the algae. Appropriate controls were also set up (Control R; containing only River water and Control E; containing only effluent from Notore without the synthetic medium). Blooms were effected by inoculating different ratios of the growth media with synthetic medium (20ml) in 500ml cotton plugged conical flask. The flasks were then incubated for two weeks under natural source of light. The flasks were intermittently shaken to enhance uniform blooming by preventing sedimentation of the microalga, improve gas

exchange between culture medium and air to ensure that all the cells were equally exposed to light and nutrients. The temperature, pH and the microalgal concentration (Optical density) of the mixed culture were determined daily for 14 days.

2.4 Isolation and Identification

The Agar plate technique was used for the isolation of the *Chlorella* sp. About 0.1ml of the bloomed culture, from the fertilizer company was plated on bacteriological nutrient agar plates with antibiotics added to prevent bacterial and fungal growth after autoclaving. The spread plate culture technique was used. The plates were incubated for 5 days, in a shade, under natural illumination (sunlight). Colonies which appeared after 4 days were subcultured to get a pure culture. The colonies were identified using cultural and morphological characteristics. Inocula of about 20ml were prepared with sterile distilled water and the pure *Chlorella* sp. colonies.

2.5 Flue Gas Trapping

The Flue gas was trapped from a TEC 3.0 kW gasoline generator, with a 1000ml Duran bottle that contained 0.1M NaOH solution, after which the solution was allowed to cool before checking for the physicochemical properties and CO_2 saturation.

2.6 Experimental Set Up

The basal medium used for the set up was prepared from the Notore effluent and the New Calabar River water. This was mixed with 1ml of prepared inocula and flue gas solution which served as the source of Carbon (CO_2) and other nutrients for the growth of the *Chlorella* sp. The flue gas delivered into each of 500ml conical flasks containing the liquid mixture was estimated at different concentrations viz., 2%, 5%, 10% and 15%. The set up was done in triplicates. The cultures were aerated with an aquarium pump supplying air at the rate of *c*a 150 bubbles per minute, connected to the flasks through rubber and plastic tubing. The flasks were corked with cotton wool to prevent contamination. Appropriate controls containing sterile distilled water and 1ml of the inocula without the flue gas solution were also set up.

2.7 Analyses

Samples were taken every 2 days for 14 days to monitor the amount of CO_2 removed as well as changes in pH and temperature. Cell density was monitored through optical density and biomass (cell dry weight and cell number). The lipid yield was determined at the end of the 14^{th} day.

2.7.1 *Chlorella* sp. biomass by optical density (OD)

The Optical Density (O.D) was determined using a spectrophotometer (Spectronic 721 model) set at 600nm. About 5ml of the growing culture were removed aseptically, placed in the cuvette after blanking and the absorbance was measured at 600nm.

2.7.2 Quantitative determination of *Chlorella* sp. growth

The biomass, as dry weight, was measured by harvesting 10ml of growing culture by centrifuging at 3000rpm for 15mins. The cells were washed 3 times with physiological saline and dried at 50ºC in a hot air oven until a constant weight was obtained with a (RAGWAG AS/22) weighing balance. The cell number was counted using the Improved Neubauer haemaocytometer counting chamber. About 1ml of the culture was diluted tenfold. A clean Neubauer coverslip was charged to the counting chamber by pressing it carefully and firmly in place. Then 0.02ml of the diluted sample was collected with a clean Pasteur pipette and gently allowed to run quickly through the edge of the cover slip into the chamber, until it was well spread. Thereafter the chamber was allowed to stand for at least two minutes before counting using the Leitz light microscope. The grids under the microscope were examined using x10 objective and later refocused at x40 before counting the cells in the 4 large squares.

2.7.3 Lipid extraction

The lipid extraction of Kates [18] was adopted. The wet cell paste containing about 40mg of cells (dry weight) was diluted to 1ml with sterile distilled water in a 15ml glass stoppered centrifuge tube, to the suspension in the centrifuge tube was added 3.75ml of methanol-chloroform (2:1, v/v); the mixture was shaken and left at room temperature for 2 hours with intermittent shaking. After centrifugation, the supernatant was decanted into another 15ml glass stoppered centrifuge tube, using a Pasteur pipette the residue was re-suspended in 4.75ml of methanol-chloroform-water (2:1:0.8), the mixture was then shaken and centrifuged. To the combined supernatant was added 2.5ml each of chloroform and water, and the mixture centrifuged. The lower chloroform phase was withdrawn, diluted with 10ml benzene (to aid the removal of traces of water) and brought to dryness in a rotary evaporator (30-35ºC), leaving the lipid which was then weighed.

2.7.4 Determination of CO_2 saturation

The double indicator-titration method [19] was used.

2.8 Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) was used to calculate the mean and Standard Deviation (SD). One-way ANOVA was used to test for significant differences between treatments. All used statistics were based on 95% confidence level.

3. RESULTS AND DISCUSSION

3.1 Algal bloom

Flasks showing different ratios of (E: R: S) before blooming was initiated and their controls; (E) and (R) is shown in (Plate 1). There was effective blooming under natural light within 5-8days (Plate 2). The result shows that the effluent and river water complemented each other. The controls did not show any sign of blooming.

Plate 1. Flasks showing different ratios of (E: R: S) before blooming was initiated and their controls; (E) and (R)

Plate 2. Flasks showing algal blooms of different ratios (E: R: S) and their respective controls (E) and (R)

KEY
E = Notore effluent
R = River water
S = Synthetic medium.

3.2 Microalgae Concentration (Optical Density) in the Mixed Culture

The cell density (OD 600nm) of the mixed culture of microalgae is presented in Figure 1. The optical density showed a distinct lag phase of about 4days, followed by exponential phase (between 5 – 8days), then stationary phase between 8 – 10days and then the death phase gradually occurring between 11 – 14 days. This figure thus depicts a complete growth curve.

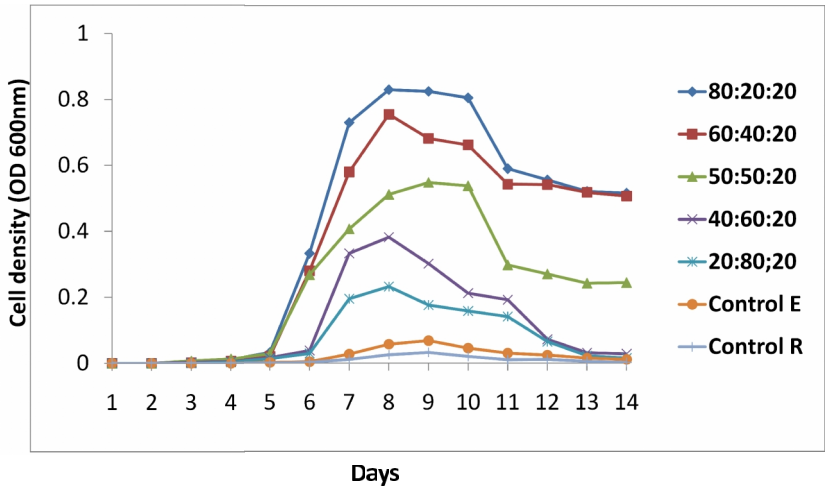

Figure 1: Cell density (OD 600nm) of blooming of the microalgae as mixed culture using Notore effluent, River water and synthetic medium in different ratios.

KEY
E = Notore effluent
R = River water

3.3 Algal Isolates

Rapid growth of *Chlorella* sp. was observed after four (4) days of inoculation. The colonies appeared grass green, slightly raised and oval in shape. A single colony of the *Chlorella* sp. was streaked to isolation until a pure culture was obtained. The microscopic view of a single colony of *Chlorella* sp. showed a single green alga cell, solitary, very small (3-3.5µm) and globular or ellipsoidal. The cells were surrounded by a thin cellulose wall which enclosed a cup shaped chloroplast. The cells were devoid of flagella and contractile vacuoles but contained a centrally located nucleus (Plate 3).

The use of nutrient agar is therefore an added advantage compared to the normal medium, (BG 11). Besides its high cost and difficulty in its formulation, BG 11 medium will give similar growth results as the agar within 3weeks. Several researchers [20, 21, 22, 23] have used nutrient agar plates for isolation and purification of *Chlorella*. Elimination of bacteria and fungi were verified after plating the microalga on an antibiotic free nutrient agar. The absence of any further contamination could be attributed to the presence of the pigment, chlorellin, which is produced by *Chlorella* [24].

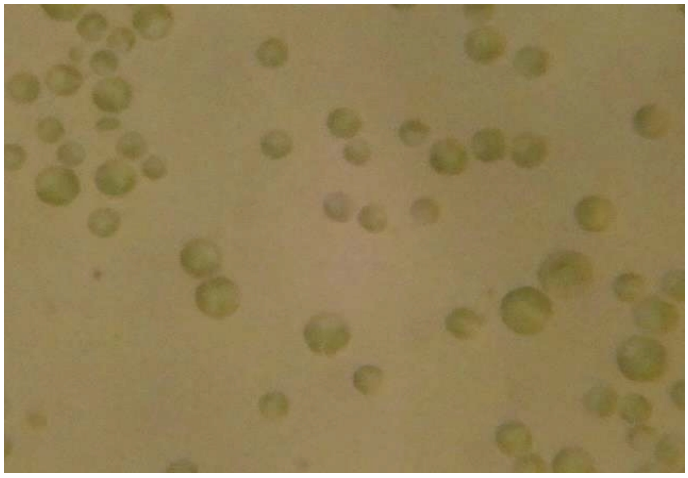

Plate 3. Microscopic view of *Chlorella* sp. isolate as seen under the light microscope (X40 Magnification).

3.4 Physicochemical Properties of the Formulated Growth Medium

The physicochemical properties of the trapped flue gas solution, the Notore effluent and the New Calabar River water are presented in Tables 1 and 2, the flue gas solution had high nitrate and sulphate content. This agrees with the previous work by Olaizola [25] that in flue gas, sulphate is usually the highest of all the nutrients after CO_2 followed by nitrate. Anaga and Abu [24] also reported that the ions are necessary for the stimulation of microalgal growth causing good blooming of the organisms. The flue gas solution gave no lag phase (Figure 2) compared to the other media formulations (Figure 1) that gave a lag phase of 4 days.

Table 1. Physicochemical properties of water sample from Notore chemical company (NCC) and the New Calabar River (NCR)

Properties	NCR	NCC
Temperature(^{o}C)	31.2	29.1
pH	7.4	7.5
Total nitrogen(mg/l)	0.07	5.9
Nitrate(mg/l)	1.147	2.7
Phosphate(mg/l)	0.008	4.27
Sulphate(mg/l)	6.889	4.822
Ammonia(mg/l)	0.14	3.3
Total Organic Carbon(TOC)mg/l	0.0345	9
Total Dissolved Solids(TDS)mg/l	20	107
Conductivity(uS/cm)	36	228
Chloride	12	10.6
Iron(mg/l)	0.07	0.23
Zinc(mg/l)	0.05	0.8

Table 2. Main physicochemical parameters of components used in the formulation of media for the growth of *Chlorella* sp. on flue gas

Parameters	Notore	River	Flue gas solution
pH	7.5	7.4	9.2
Nitrate(mg/l)	2.7	1.147	4.412
Sulphate(mg/l)	4.822	6.889	1033.335
Phosphate(mg/l)	4.27	0.008	0.0165

3.5 Cultures of *Chlorella* sp. Grown on Flue Gas

The flue gas solution was found to support the growth of *Chlorella* sp. This was indicated by the increase in chlorophyll (increase in green colour of the culture) produced from day 2 to day 10. A decrease in chlorophyll was however noticed from day 12. The decrease in chlorophyll was first noticed in the 2% concentration followed by 5%, 10% and 15% concentrations. Plates 4 and 5 shows the cultures on day (0) and (14) of the cultivation period. The *Chlorella* sp. was found to grow within a temperature range of 29±2^{o}C. The pH varied considerably. The cultures started with a slightly alkaline pH of 7.8, 7.9, 7.9 and 7.8. On the 2nd day, the pH changed to 8.0, 8.1, 8.3, and 8.3. The pH continued to rise till the 8th day, when it attained an optimum pH of 8.7, 9.3, 9.4 and 9.6 and decreased again finally on the 14th day to 7.6, 8.7, 9.2 and 9.5 for the 2%, 5%, 10% and 15% flue gas concentrations respectively.

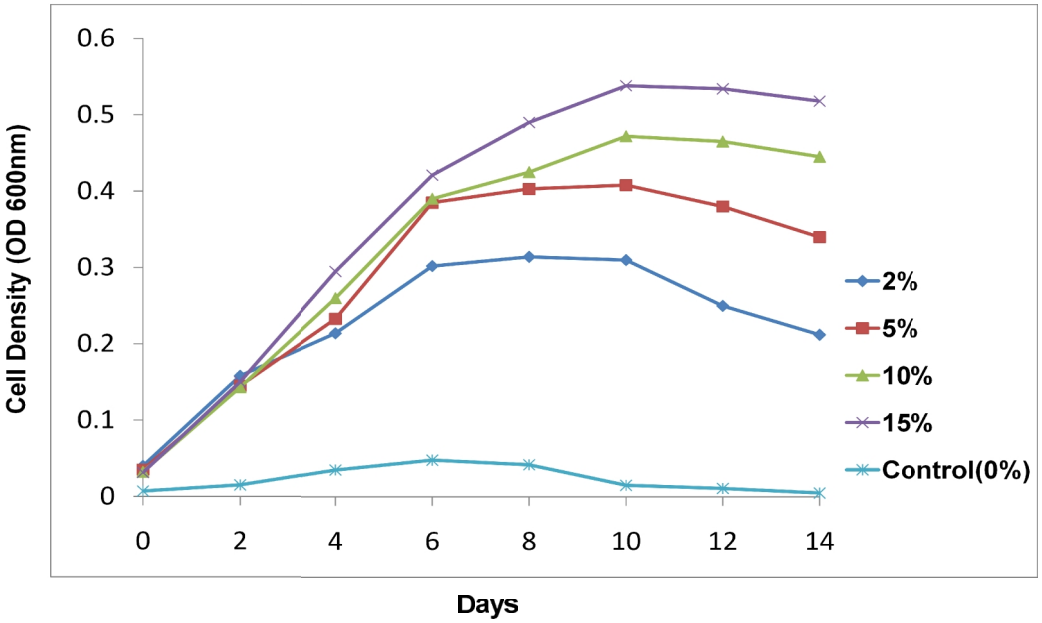

Figure 2: Growth of *Chlorella* sp. on different concentrations of flue gas measured as Optical density (OD 600nm).

Plate 4. Growth of *Chlorella* sp. on different concentrations of flue gas – Day (0)

Plate 5. Growth of *Chlorella* sp. on different concentrations of flue gas – Day (14)

Generally carbon source for autotrophic growth of microalgae is CO_2 from the atmosphere which is naturally present at approximately 300ppm. Microalgae have a high growth rate and CO_2 fixing ability. So one alternative way, according to Qiang and Milton [27], to capture the CO_2 emitted from industries is by alkali absorption and storage in liquid as HCO_3^- and CO_3^2.The use of NaOH to capture flue gas will help to maintain a neutral pH due to lowering culture pH by dissolved NOx and SOx [28]. In this study, the flue gas was captured with 0.1M NaOH to form a solution, with a pH of 9.2.

The flue gas solution served as a source of carbon and other nutrients (Table 2) for the microalga and it gave no lag phase (Figure 2). This was indicated by the changes in the various parameters measured; The cell number as shown in Figure 3 was found to increase from 1.0 $x10^7$, 1.5 x 10^7, 1.0x 10^7 and 1.5 x 10^7 to 7 x 10^7, 1.25 x 10^8, 1.50 x 10^8 and 1.7 x 10^8 cells/ml with a dry cell weight of 3.30mg/ml, 4.40mg/ml, 5.40mg/ml and 5.60mg/l (Figure 4) for the 2%, 5% 10% and 15% flue gas concentrations respectively.

Lipid yields of 45mg/g, 47.5mg/g, 52.5mg/g, and 57.5mg/g of cell dry weight were obtained on the 14th day for the 2%, 5%, 10% and 15% concentrations of the flue gas respectively (Figure 5). Chang and Yang [28] grew *Chlorella* sp. at concentrations of 1% and 15% with biomass productivity of 350± 40mg/l and 420± 50mg/l with a volumetric lipid production of 150±12mg/l. Chu et al. [29] have grown *Chlorella* sp. NTU-H15 with a maximum growth rate of 0.28g dry wt/l/d at 15% CO_2.

The CO_2 concentration in the media was found to reduce. Initially the concentration of the CO_2 was at 0.0431g/l, 0.1225g/l, 0.2114g/l and 0.3244g/l for the 2%, 5%, 10% and 15% saturations respectively. The reduction (Figure 6) suggests that the microalgae utilized the CO_2 for their photosynthetic activities, converting it into algal biomass [27].

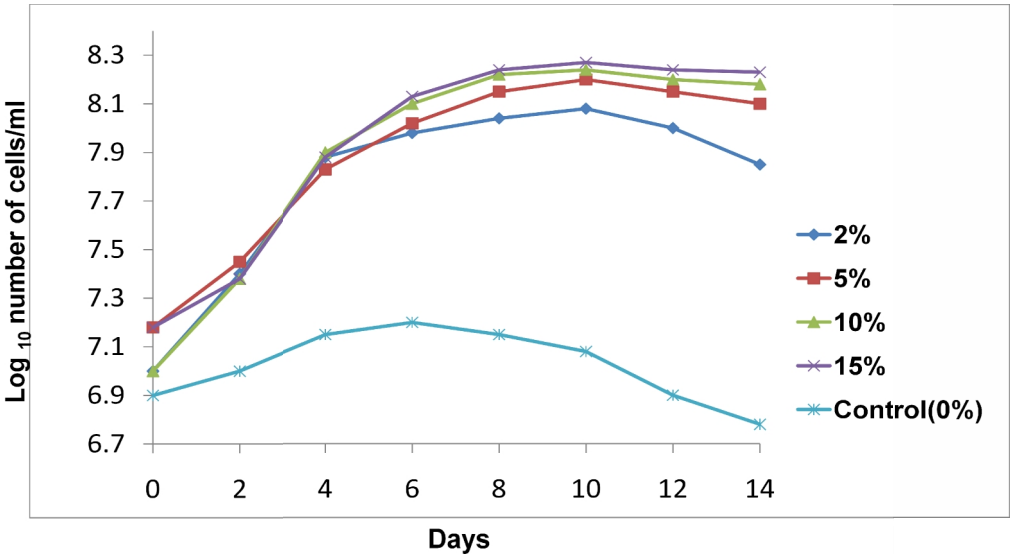

Figure 3 : Growth of *Chlorella* sp. on different concentrations of flue gas monitored by cell number.

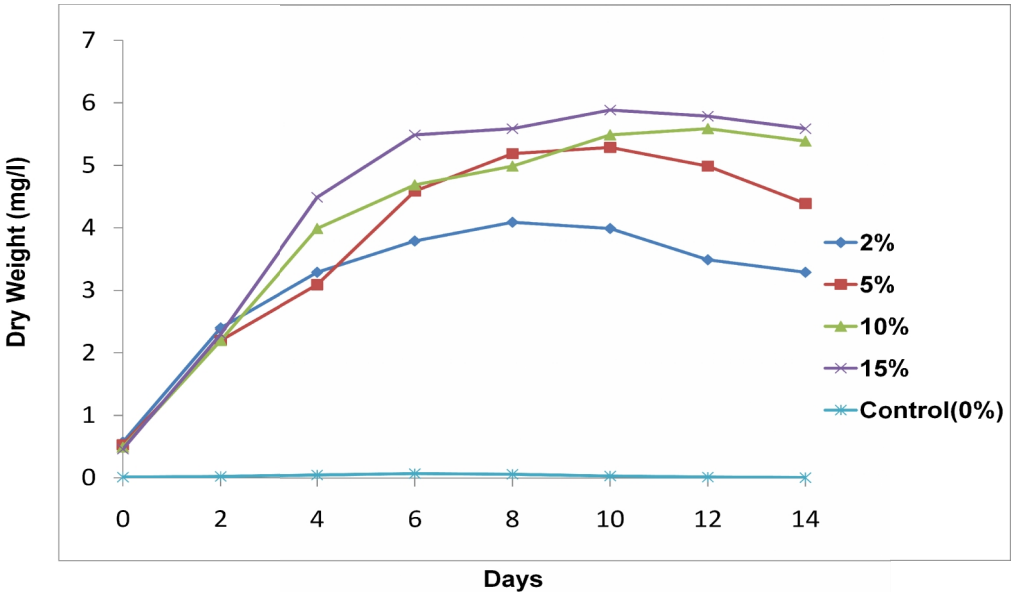

Figure 4: Growth of *Chlorella* sp. on different concentrations of flue gas measured as dry weight (mg/l).

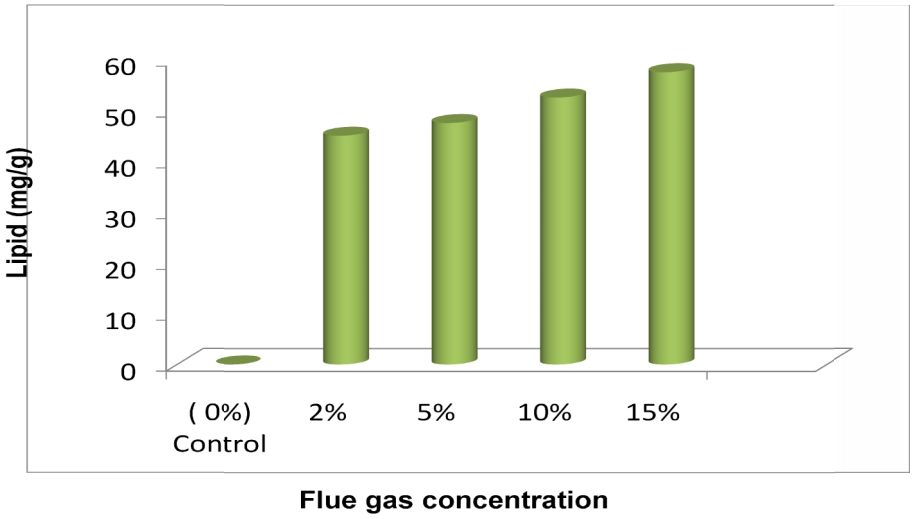

Figure 5: Lipid yield(mg/g) of *Chlorella* sp. grown on different concentrations of Flue gas .

Through the photosynthetic machinery, the *Chlorella* sp. cells fix CO_2 and convert it into organic macromolecules (carbohydrate, lipids, and proteins) stored in the cell. Statistical analysis using the one-way ANOVA revealed a significant difference for optical density, dry weight, pH, cell number, CO_2 removal by *Chlorella* sp. within the groups measured at 95% confidence level (*P*= .05).

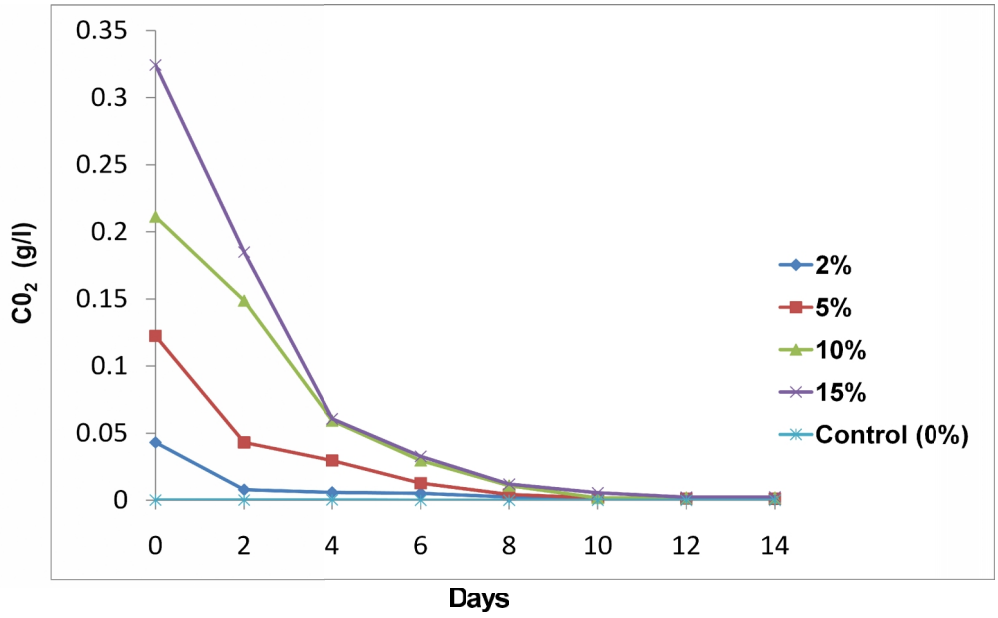

Figure 6: Removal of $C0_2$ by *Chlorella* sp. grown on different concentrations of flue gas.

In all the growth parameters measured the 15% flue gas concentration gave the highest value. Chang and Yang [28] grew *Chlorella* sp. with 5-40% CO_2 with a maximum growth at 15% CO_2. In contrast, Chu et al. [29] reported that 2% gave the maximum growth. Earlier studies [30] showed that *Chlorella* sp. had optimum growth at 10% CO_2. By controlling the pH changes in the culture and releasing CO_2 to the microalgae on demand, growth could be sustained even at 100% CO_2 [25]. It is believed that microalgae could tolerate high concentrations of CO_2 by adjusting their structural anatomy and re-distribution of certain organelles [31].

4. CONCLUSIONS

It is clear from this study that flue gas is a good source of carbon (CO_2) and other essential nutrients required for the cultivation of *Chlorella* sp. The *Chlorella* sp. isolate proved to be a good biological system for converting flue gas into biomass. More than 50% of CO_2 was utilized by the microalga and converted to biomass. This can reduce environmental pollution arising from emissions such as SOx and NOx. The sequestering of CO_2 would mitigate the effects of global warming. Thus, the gases which are considered as polluting waste can be channeled towards the culture of *Chlorella* sp. on a large scale thereby adding value to the flue gas. Therefore a system that couples a waste CO_2 source with the cultivation of *Chlorella* sp. will not only reduce cultivation costs (i.e does not require huge costs on transportation of CO_2 if the microalga is cultivated near the power plant) but would also sequester carbondioxide directly, thus, avoiding the costs of separation of CO_2 gas from the waste emissions. In addition, the production of lipids, protein and carbohydrates by the *Chlorella* can be exploited. Commercial applications for biochemical, food and pharmaceutical industries as well as environmental biotechnology are some of the benefits.

It is no secret that the world is running low of its reserves of fossil fuels. We must begin to wane our use of fossil fuel and begin replacing the sources from which the energy we use is created. *Chlorella* sp. is a veritable resource to the environment that could offer a solution to both dwindling oil supplies of the world and environmental pollution.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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