Optimization of Chlorella-Biomass Production Using Domestic and Restaurant Waste Water as a Potential Feedstock

K. F. Williams [a*] and O. K. Agwa [a]

[a] *Department of Microbiology, University of Port Harcourt, Rivers State, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

The quest to invent a cost-effective media for commercial cultivation of microalgae biomass has remained a cause for motivation for quite some time now. In this study domestic and restaurant wastewater were obtained from Choba, Rivers State Nigeria. The biosafety of the wastewater was ascertained and prepared as a media for the cultivation of microalgae. The samples were prepared in 180:20, 160:40, 140:60, 120:80, and 100:100 ratios using pond water and mixture of domestic and restaurant wastewater respectively. The blooming process was observed for seven days and biomass was monitored by cell optical density and dry weight. The result revealed that biosafety evaluation saw a reduction from 120cfu/ml to 0cfu/ml on the third day. The optimal wavelength selected for growth monitoring was 620nm while growth media ratio selected was 120:80 for restaurant:domestic wastewater. The optimization revealed pH 6.0, temperature 30^0C, salinity 10ppm and photoperiod 12:12 day:night as optimal condition. Domestic wastewater can be a veritable medium for cultivation of *Chlorella* sp as a means of integrated waste management; the *Chlorella* biomass can be used as a feedstock for various biotechnological applications such as source of biochemical, nutraceuticals and for use in biofuel generation.

Keywords: Chlorella-Biomass; domestic; optimization; restaurant; wastewater.

_____

*Corresponding author: E-mail: williamskayode22@gmail.com;

1. INTRODUCTION

The urgent request in clean energy supply has increase within the last six years [1]. This is because of the current growing trend in the population explosion, industrial revolution, and eco-deterioration. One of the challenges facing the world is shortage in Clean and renewable energy sources, energy supplies and the decline in the quality of the environmental conditions. The connection between these problems and their solution is not far-fetched. These current challenges suggest a vibrant fusion between industrial waste management, phycology and algal biotechnology, as one way of solving energy supply problems. The investments in the use of biological resources includes saline and freshwater sources, production of biofuels to meet growing demand and decline of non-renewable and conventional fuels [2]. Green energy is intimately connected with economic development and global stability. Examples include solar energy, either thermal or photovoltaic, hydroelectric, geothermal, wind, biofuels and carbon sequestration systems, among others (Mata et al*.,* 2012). Currently, many options are being studied and implemented with different degrees of success. The efficacy of microalgae to capture solar radiation, fast growth rate and yield, the algal potential to produce biomass without competing with food prices [3,4]. One important goal is to take measures for transportation emissions reduction, such as the gradual replacement of fossil fuels by renewable energy sources, where biofuels are seen as real contributors to reaching those goals, particularly in the short term (Mata, et al*.,* 2012). Safe energy production has necessitated the need to explore better and cheaper biomass-sourced energy of renewable as sustainable feedstock options as sources of inorganic growth nutrients with little or no greenhouse production (Demirbas, 2009; Verma et al*.,* 2010, Chisti, 2013). This biomass could be animal, microbial or plant derived. Various challenging issues facing these biomass options lie in the management of these natural resources without be-quitting a negative legacy to the future generations. Sadly, a plethora of the bio-resources have remained untapped [5]. Biomass has always been a major source of energy and is presently estimated to contribute 20–24% of the world's energy supply. Many species of microalgae have been reported to accumulate very significant amount of biomass production [3]. These biomass resource can be derived from organic matter, in which they trap energy from sunlight is stored in their chemical bonds. This biomass has served as energy reservoirs, storage materials by these algal species. Nutrient limitation, environmental stress and starvation have been attributed to high biohydrogen production while the reverse seem to favour biomass accumulation [6,7], Mata et al*.,* 2012, Chisti, 2013. As an energy source biomass can either be used directly via combustion to produce heat, or indirectly after converting it to various forms of biofuel. They might as well be used as a possible replacement for other competitive resource and issues created by the conventional fuels available, it can be processed into electricity, fuel and heat (Antoni et al*.,* 2007). Conversion of biomass to biofuel can be achieved by different methods which are broadly classified into: *thermal*, *chemical*, and *biochemical* methods. The advantages of using biomass could range from reduction in green-house gas emissions to reduction in over reliance on fossil fuel; it would support an integrated waste management to agro-based industry and create wealth. This wide-ranging potential for biomass and its associated products has been described as the 'bio-refinery' concept [3], Ehimen et al. [20].

2. MATERIALS AND METHODS

2.1 Algal Sample Isolation

The pond water samples were enriched with domestic: restaurant (60:40) bloomed, the selection of the *Chlorella* sp was based on its morphological features, then the strain was isolated using 100 µg/ml chloramphenicol and 62.5 µg/ml nystatin using a solidified BG11 medium Farahani et al. [8].

2.2 Identification of the *Chlorella* sp.

After blooming, characteristic colonies (round green) were picked from the broth and purified by serial subcultures. The pure culture was harvested by flooding and preserved in the refrigerator. The purity of the culture was monitored by regular observation under the microscope. The isolated microalgae were identified microscopically using the light microscope at x40 ocular with a standard manual for algae (Shashikant and Gupta, 1998).

2.3 Determination of Optimal Wavelength

The optimal wavelength of the medium was obtained by scanning the waste water (effluents) from a low to high wavelength of the

spectrophotometer. The optimal wavelength was determined from the point of least absorbance [9,10].

2.4 Process Optimization (Light and Dark Phases) for Selection of Biomass Production

2.4.1 Substrate ratio

The ratio of the domestic wastewater and restaurant wastewater was used to ascertain best ratio of the two. While the novel synthetic media was used as positive controls and the un-inoculated waste water was used as negative controls.

2.5 Interaction of Operational Factors

The methods of Ogbonda et al, [11] was used in determining the interaction between the microalgae and sewage concentrations and operational factors under the dark and light phases. The pH (Olguin, 2003; Griffith et al., 2009; Al-Safaar et al., 2016), temperature (Serra-Maia et al., 2016), salinity (Salama et al., 2013), and photoperiod 12:12 and 6:18 [11] were used to detect optimal points by the sigmoid graphs.

2.6 Effect of Photoperiod on Chlorella Biomass

The methods of Ogbonda et al., [11] was used in determining the effect of photoperiod on *chlorella* biomass growth. The florescent tube under light intensity of 12:12 and 6:18 photoperiod. The cell dry mass and optical density was determined.

2.7 Effect of Salinity on *Chlorella* Biomass

The modified method of Salama et al., (2013) was used in determining the effect of salinity on *chlorella* biomass growth. The % NaCl 2%, 3%, 4%, 5%, 6%, 7%, and 8%, was weigh and dissolved in 100ml of the medium each. The medium was sterilized for 121^oC at 15minutes and allowed to cooled 1% of the chlorella cell was inoculated. The aquarium pump was connected to pump in oxygen into the setup. The cell dry mass and optical density was determined.

2.8 Effect of pH on *Chlorella* Biomass

The pH meter was calibrated using buffer 7.0 and the media pH levels were adjusted at specific ranges using 1.0N HCl and 1.0N NaOH (Olguin, 2003; Griffith et al., 2009; Al-Safaar et al., 2016). Studies have shown that minor fluctuations in the pH could affect growth rate of microalgae. The pH of the media was adjusted to 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 and growth was monitored at these pH conditions. One percent of the *chlorella* cell was inoculated; day 0 to day 14 of the experimental setup each was taking 10 ml for cell dry mass and optical density to determine the *chlorella* growth.

2.9 Effect of Temperature on *Chlorella* Biomass

The modified method of Serra-Maia et al., (2016) was adopted in the determining the effect of temperature on *chlorella* biomass production. The temperature 20^0C, 30^0C and 35^0C was considered in this study. The medium contain 100ml and 1% *chlorella* cell was inoculated in each of the temperature range in the experimental setup. One percent of the *chlorella* cell was inoculated; day 0 to day 14 of the experimental setup each was taking 10ml for cell dry mass and optical density to determine the *chlorella* growth.

2.10 Biomass Yield Studies and Monitoring

2.10.1 Determination of optical density

The method for Agwa et al*.,* [8] was adopted in the determination of the optical density of the growth of the microalgae in media. The optimum wavelength was used to monitor the growth of the microalgae in the sewage. About, 10ml of the broth was poured into a cuvette and the absorbance read-off from the spectrophotometer.

2.11 Determination of Cell Biomass

The Cell Dry weight approach was used to determine the algal biomass yield. Ten milliliters (10ml) of the culture broth of the blooming set up was transferred to a centrifuge tube and centrifuged at 4,500xg for 15 minutes three times, the pellets were dislodged and then poured on a pre-weighed Whatman filter and the sample was dried at 50^oC hot oven to constant weight 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 [12,8,13].

2.12 Data Analysis

The response surface methodology (RSM) was use to compare the optimal points of significance at a confidence level of 95%.

3. RESULTS

3.1 Wavelength for Monitoring Growth

The results demonstrate a corresponding fall in the absorbance of the formulations as the wavelength increased. The wavelength was increased manually from 500-820nm, the corresponding absorbance level fell from 0.9nm to 0.01nm. The process was repeated for the restaurant and domestic wastewater for increased precision in the selected wavelength for the biomass monitoring and optimization investigations. The difference in the growth pattern for the positive control and the optimal growth point is obvious in the graph presented.

3.2 Optimized Conditions for *C. vulgaris* Growth

3.2.1 Effect of temperature on *Chlorella* biomass accumulation

The effect of temperature variations on the biomass production, accumulation of cell biomass and the optimal conditions for the biomass production. The results present the growth pattern of the 30°C and the positive control had a lag phase between the first day. The negative control which was uninoculated with the 3-day old culture of the *Chlorella* had no significant increase. The 20 and 35 ^{o}C had a no lag phases and had obvious greening of the medium after the exponential increase of the cell suggesting the presence of soluble nutrients to the algal cells at the temperature levels tested. The biomass accumulation as a measure of the cell dry weight of the feedstock.

3.3 Effect of Salinity on *Chlorella* Biomass Accumulation

The response of *Chlorella* sp to saline environments. The best growth was observed for the 10 ppm of sodium chloride, the log phase started from the day 0 to day 2, the 0.65 to 1.12 Abs. The positive control had a lag phase for the phase first 24h and an exponential phase for the first 1h 30 minutes. Also the 15ppm had also a high biomass growth, the negative control remained unchanged for the entire growth period. Similarly other salinity levels had different but not significant. The statistical analysis using two-way ANOVA revealed that there were no significant changes in biomass accumulation. Fig. 4 describes the accumulation of biomass as a measure of cell dry weight, the lag phase for all the tested levels of the salinity but 25ppm had no lag phase, again the 10 ppm had an obvious increased or even doubled the biomass produced within the first 2 days.

3.4 Effect of pH to *Chlorella* Biomass Accumulation

The response of the feedstock to varying level of the pH ranges. The results suggest that the pH 6.0 and pH 6.5 had a better biomass accumulation with the lag phase of between 0-3days. Biomass accumulation pH 7-8.5 had no striking significant difference. The negative control set up had no growth as the absorbance remained unchanged. The positive control which had a 24h-old lag phase had a growth curve which crisscrossed the negative control, which suggests the growth out did the density of the uninoculated medium with a 3-day old exponential phase. The cell dry weight for the pH 6 showed a significant change in the amount of biomass accumulated, from the positive control, as seen in Fig. 4. The other pH levels did not show any significant biomass. The biomass accumulation as seen by the cell dry weight for the positive control and pH 9.0 had the lowest cell dry weight.

3.5 Effect of Photoperiod on *Chlorella* sp. Biomass Accumulation Using Domestic and Restaurant Wastewater

The growth pattern of the isolates after their prior exposure to the 12:12 and 6:18 photoperiod for the blend of the wastewater, the study suggest the former photoperiods had a significant response compared to the negative and the positive control. The study observed an increase of the population of the cells with a log/exponential phase between 0-3days. The graph also described the inability of the cells to accumulate significant amount of algal dry weight of cells were observed to increase in a geometric manner within the first 5days of monitoring. Statistical analysis using one-two way ANOVA and least significant difference (LSD) showed that there is the photoperiod affected the accumulation of biomass at p-value <0.05. The positive control were observed to have corresponding rise in biomass but performed least.
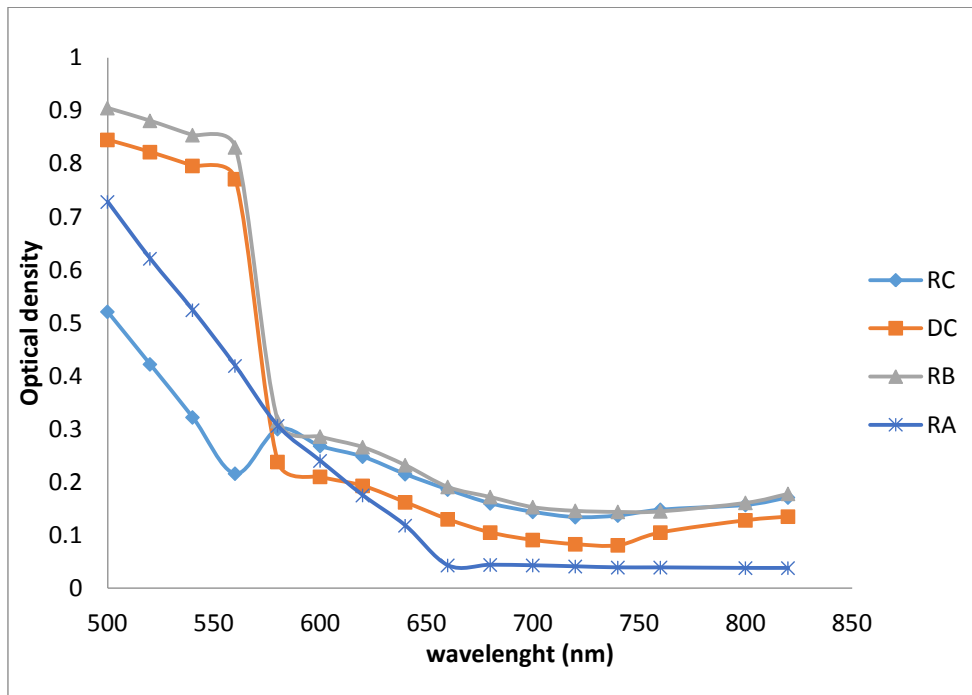
Fig. 1. Wavelength selection for restaurant and domestic wastewater
Key: RC=Restaurant C
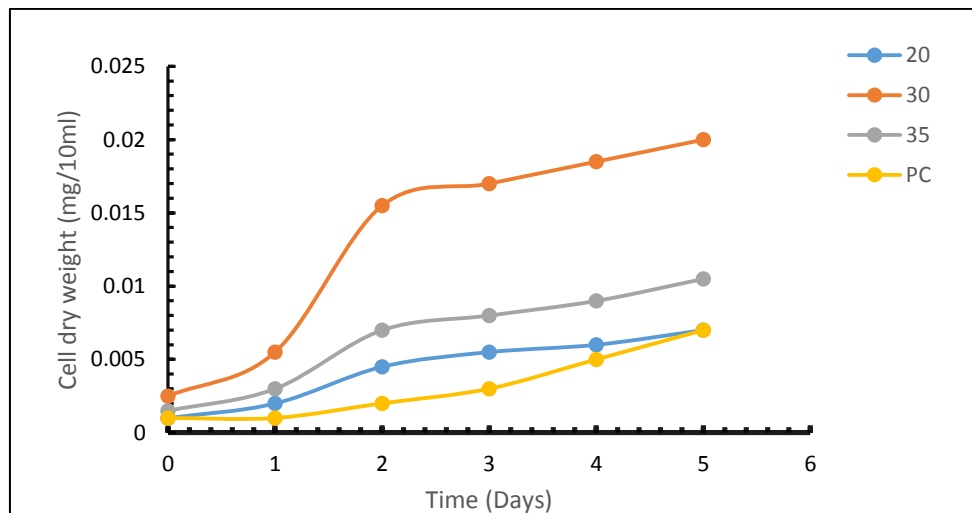*DC= Domestic C*
RB= Restaurant B
RA= Restaurant A

Fig. 2. Effect of Temperature on algal biomass using a mixture of restaurant and domestic effluent
Key: PC= Positive Control
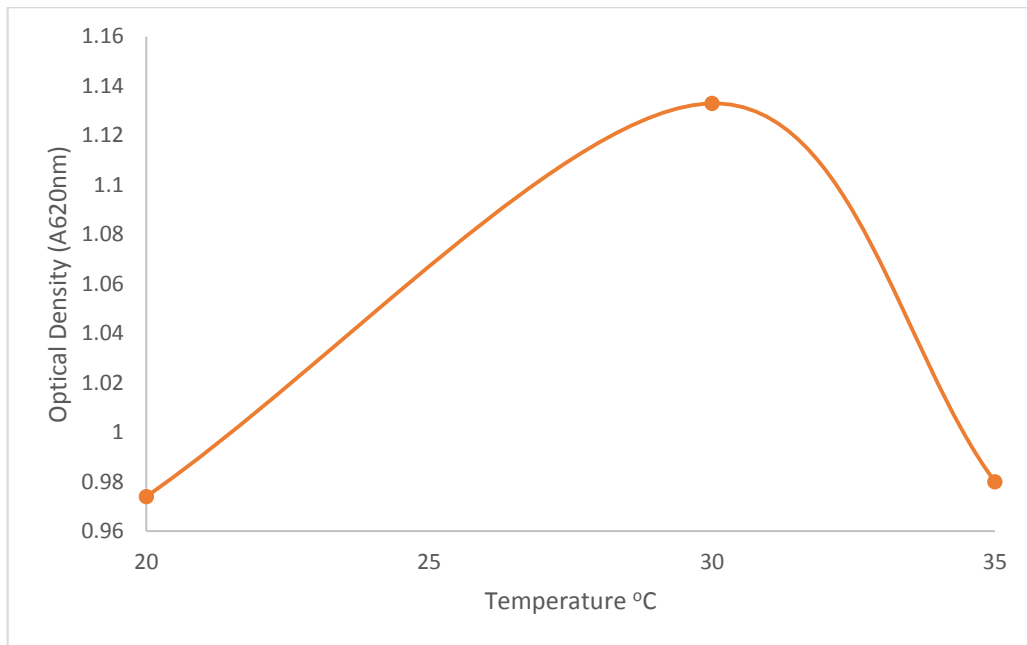*Temperature = 20^0C*
Temperature =30^0C
Temperature =35^0C
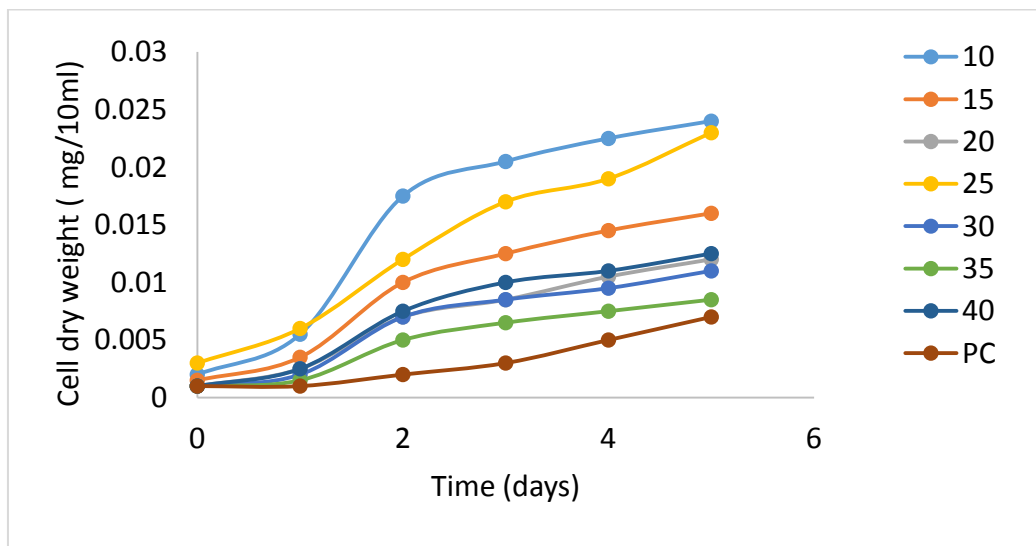
Fig. 3. Optimal temperature selection

Fig. 4. Effect of salinity on *Chlorella* biomass using a mixture of restaurant and domestic effluent

Key: PC= Positive Control
10/5= 2% NaCl
15/5= 3% NaCl
20/5= 4% NaCl
25/5= 5% NaCl
30/5 = 6% NaCl
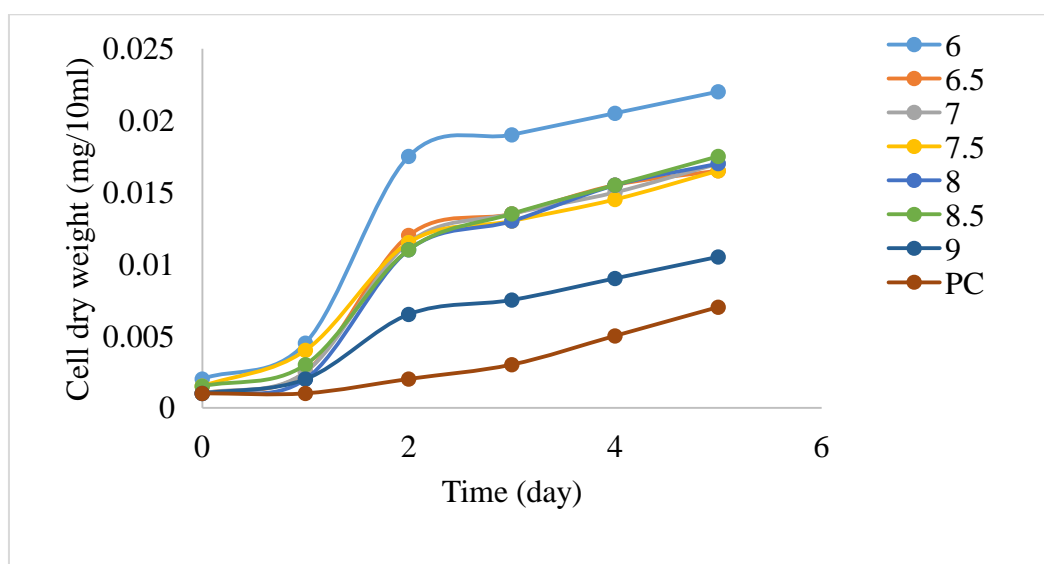*35/5 = 7% NaCl*
40/5= 8% NaCl

Fig. 5. Effect of pH on *Chlorella* biomass using a mixture of restaurant and domestic effluent
Key: PC= Positive Control
pH = 6
pH = 6.5
pH = 7
pH = 7.5
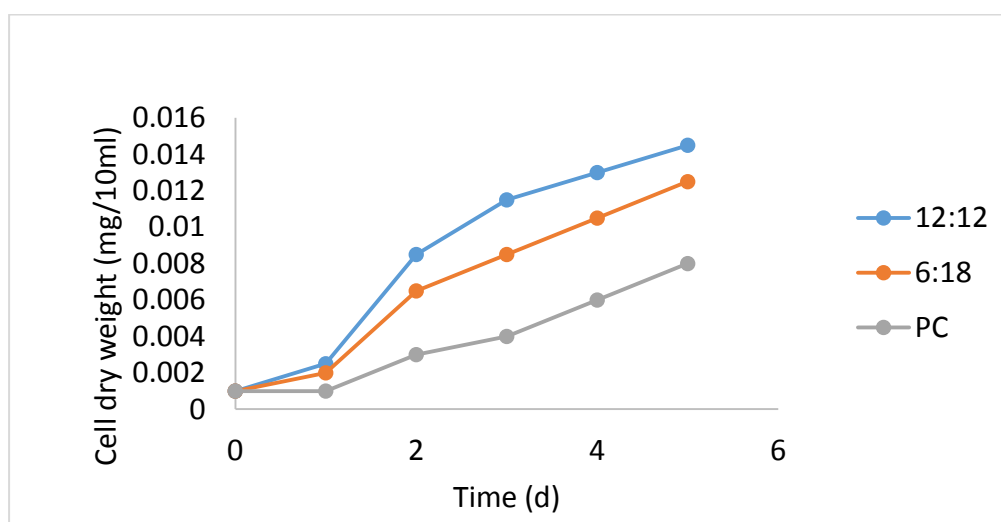*pH = 8*
pH = 8.5
pH = 9

Fig. 6. Effect of photoperiod on the cell dry weight
Key: PC= Positive Control
Photoperiod = 12:12
Photoperiod = 6:18

4. DISCUSSION

4.1 Optimal Conditions for Microalgae Growth

The growth composition of the domestic and restaurant wastewater for the cultivation microalgae was determined. The wavelength was increased manually from 500-820nm, the corresponding absorbance level fell from 0.9nm to 0.01nm. The process was repeated for the restaurant and domestic wastewater for increased precision in the selected wavelength for the biomass monitoring and optimization

investigations. The difference in the growth pattern for the positive control and the optimal growth point is obvious in the graph. This is similar with Effiong et al, (2019). The growth performance of the different ratios of the restaurant and domestic wastewater 120:80 which is also equivalent to the 60:40 for the restaurant wastewater (RW) and domestic wastewater (DW) respectively for the different samples tested. The entire growth formulation of the substrate describes the substrate ability to support the growth of the algae. This is in line with Effiong et al, (2019).The temperature is a crucial factor in the cultivation of microalgae. The extremes of the factor could harm or deter the growth of algae [14]. In this study, the optimal temperature recorded with the wastewater was $30^{o}C$. The results of this study agrees with the findings of Cho et al. [14] who reported the same temperature. The work of Hurs et al., 2008 reported that high temperature does not favour maximum growth activity in *Chlorella ellipsoidea*. Earlier findings of Huang and Rorrer (2002) reported an optimum temperature of $24^{o}C$. The extremes of the temperature retard the biomass productivity of the isolates. Al-Qasmi et al., [15] reported that propagation of cells were at their peak between $27-31^{o}C$ and bioconversion of organic carbon sources were also efficient at such conditions and may account for the rise in biomass accumulation as seen in Figs. 2 and 3. The Salinity is a measure of ions and salts present in a medium, the report of Alkhamis and Qin [16] reported that levels of salinity affected the growth of cells. In this study, lower levels of salinity affected biomass quality as higher biomass were obtained at lower salt concentrations as seen in Fig. 4. The work of Cho et al. [14] suggested that an optimum salinity of 10ppm was recorded. The response also favoured more biomass accumulation on the basis of the cell dry weight. Salinity levels did not affect the specific growth rate (SGR) of feedstock. The pH of the medium provides information on the absorption and bioavailability of nutrients available to the feedstock to cultivate microalgae. In this study, the optimal pH was observed to be 6.0, this agrees with the reports of Pandey and Tiwari [17]. They reported that pH tolerance of microalgae is specific for algae-type and reported that pH 9.0 is critical for the growth of *Spirulina maxima* contrary to the 7.5 reported to support *Chlorella* sp [8]. The optimal conditions also suggest a possible high throughput of biomass. The determination of the photoperiod is a crucial procedure in the selection of media activity, the ratios of the day and light regimes were critical to the growth of microalgae. The result of this study agrees with the report of Mata et al. [18] that 12h day and light encouraged the production of biomass more. This also agrees with the report of Jacob-Lopes et al. [19] who asserted that 24 h dark shuts down the production of algae biomass. Photosynthetic efficiency of the cells halted at extreme dark and light conditions. The findings of this report suggest that biomass were more accumulated in the 12:12 than 6:18 periods.

5. CONCLUSION

The importance of domestic and restaurant waste water as a source of essential nutrients for the cultivation of *Chlorella* sp has been demonstrated in this study. Thus, these wastes could be channeled towards the culture of these organisms on a large scale to generate high biomass for production of biofuel. This process of *Chlorella* cultivation can be considered as a renewable inexpensive resource and effective waste utilization for the growth of these microalgae. The large scale production will enhance biotechnological applications such as bioenergy production, feed, and biochemical production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Batista AP, Ambrosano L, Graça S, Sousa CP, Marques ASS, Ribeiro B, Botrel EP, Neto PC, Gouveia L. Combining urban wastewater treatment with biohydrogen production – An integrated microalgae-based approach; 2015.
2. Agwa OK, Ibe SN, Abu GO. Assessment of cow dung waste for the laboratory cultivation of Chlorella for lipid production. Astan Journal of Microbiology and Biotechnology of Environmental Science. 2012a;14:1-6.
3. Chisti Y. Research review paper: Biodiesel from microalgae. Biotechnology Advances. 2007;25:294–306.
4. Feng Y, Li C, Zhang D. Lipid production of chlorella vulgaris cultured in artificial wastewater medium. Bioresource Technology. 2011;102:101-105.

5. Zhang T. Lu, H, D., Taili, C, Niu X, Li B, Zhang D, Zhang Y. A strain of Chlorella sp. was used for chicken manure fermentation broth treatment and bio-crude oil feedstock production. Materials Research. 2014; 955-959;2714-2720.
    DOI:10.4028/www.scientific.net
6. Guschima IA, Harwood JL. Lipids and lipid metabolism in eukaryotic algae. Progress Lipid Research. 2006;45:160-186.
7. Yoo C, Jun SY, Lee JY, Ahn CY, Oh HM. Selection of microalgae for lipid production under high levels carbon dioxide. Original Research Article. Bioresource Technology. 2010;101(1):S71-S74.
8. Agwa OK, Abu GO. Utilization of poultry waste for the cultivation of Chlorella sp. For biomass and lipid production. International Journal of Current Microbiology and Applied Sciences. 2013; 3(8):1036-1047.
9. Mogany T. Optimization of Culturing Conditions and Extraction Method for Phycocyanin Production from a Hypersaline Cyanobacterium. Student thesis, Durban University of Technology; 2014.
10. Wang B, Li Y, Wu N, Lan C. Carbon (iv) oxide bio-mitigation using microalgae. Applied Microbiology and Biotechnology. 2008;79(5):707–718.
11. Ogbonda KH, Aminigo RE, Abu GO. Infuence of temperature and pH on biomass production and protein biosynthesis in a putative Spirulina sp. Bioresource Technology. 2007;98:2207–2211.
12. Fuentes-Grünewald C, Garcés E, Alacid E, Rossi S, Camp J. Biomass and lipid production of dinoflagellates and raphidophytes in indoor and outdoor photobioreactors. Marine Biotechnology. 2013;15(1):37-47.
13. Cheirsilp B, Torpee S. Enhanced growth and lipid production of microalgae under mixotrophic culture condition: effect of light intensity, glucose concentration and fed-batch cultivation. Bioresource Technology. 2012;110:510-516.
14. Cho SH, Ji SC, Hur SB, Bae J, Park IS, Song YC. Optimum temperature and salinity conditions for growth of green algae Chlorella ellipsoidea and Nannochl-oris oculata. Fisheries Science. 2007; 73(5):1050–1056.
15. Al-Qasmi M, Raut N, Talebi S, Al-Rajhi S, Al-Barwani T. A review of effect of light on microalgae growth. In Proceedings of the World Congress on Engineering. 2012; 1:4-6.
16. Alkhamis Y, Qin JG. Cultivation of isochrysis galbana in phototrophic, heterotrophic, and mixotrophic conditions. BioMedical Research International. 2013; 9:1-9.
17. Pandey JP, Tiwari A. Optimization of biomass production by *Spirulina platensis*. Journal of Algal Biomass. 2010;1(2):20-32.
18. Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications. Renewable and Sustainable Energy Review. 2010;14: 217–232.
19. Jacob-Lopes E, Scoparo CHG, Lacerda LMCF, Franco TT. Effect of light cycles (night/day) on CO_2 fixation and biomass production by microalgae in photobioreactors. Chemical Engineering Process. 2009;48:306–310.
20. Ehimen EA, Connaughton S, Sun Z, Carrington GC. Energy recovery from lipid extracted, transesterified and glycerol codigested microalgae biomass. GCB Bioenergy. 2009;1(6):371-381.