



(RESEARCH ARTICLE)



Temperature and photoperiods optimization for the cultivation of *Chlorella vulgaris* for growth and biomass production from cassava wastes

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Abstract

Microalgae biomass is widely being used as a third generation biofuel because they are the most promising renewable feedstock for biofuel production. Its use is greatly considered as it has high growth rate, photosynthetic efficient, not competing for arable land, efficient carbon dioxide fixation and potential to accumulate high amount of carbohydrate. Microalgae carbohydrates are contained in their cell wall mainly as cellulose and plastids as starch which are readily converted into fermentable sugars. The cultivation of *Chlorella vulgaris* on cassava wastes for growth and biomass production depends on factors such as temperature and photoperiods. Cassava waste mixtures were cultivated on *Chlorella vulgaris* stock culture at different concentration ratio at pH of 6.5 and salinity of 10mg/l, cultivation ratio of 160:40 for cassava peel water to cassava waste water CP: CW at 670nm absorbance for 7 days. Temperature variations of 20°C, 30°C and 35°C were checked to determine the optimum temperature for the growth and biomass production of *Chlorella vulgaris* on the optimum cassava waste mixture concentration. Photoperiod variation of 12:12, 6:18 and 18:6 were checked to determine optimum photoperiod for the growth and biomass production of *Chlorella vulgaris* on the optimum cassava waste mixture concentration. From the results obtained in this optimization study, optimum growth and biomass was recorded at temperature of 30 °C. Photoperiod of 12:12 was responsible for optimum growth while photoperiod of 18:6 light and dark conditions gave high biomass production. This study shows that optimized conditions are necessary high growth and biomass production of the microalgae on cassava waste.

Keyword: Microalgae biomass; *Chlorella vulgaris*; Temperature; Photoperiod; Cassava waste

1. Introduction

Photosynthetic biomass-based fuels are widely considered as viable contenders as sustainable alternatives to fossil fuels. Microalgal biomass is potent for anaerobic conversion as it can have a high content of lipids, carbohydrates and proteins and does not contain recalcitrant lignin [6]. Microalgae can lead to the production of lipids, protein and starch from photosynthetic processes that utilize light and nutrients. The relative measures of these metabolic segments are solidly associated with natural and supplement conditions including: the sum and power of daylight; CO levels; pH; temperature; accessible supplements; and, the appearance or non-appearance of organisms. The biochemical composition of the microalgal cells are adversely affected by environmental conditions such as light and temperature, the availability of non-mineral nutrients, macronutrients, and micronutrients, [7]. The cell metabolism of microalgae is also affected by other factors like, pH and the presence of poisonous metals. Generally, these elements can influence photosynthesis, therefore adjusting carbon fixation and the assignment of carbon into various sorts of macromolecules. Thus, the cell's macromolecular arrangement decides its convenience in biofuels generation. Light are the main requirement for microalgae growth and the energy source for photoautotrophic growth stage since it required in the conversion of carbon dioxide to organic compounds mostly sugars. Exposing microalgae to high light intensity will result in light constraint for the surface layer microalgae because it cannot use ingest each of the light photon available. Photo inhibition phenomenon which retards microalgal growth rate occurs as a result of the continuous increase in light

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intensity level. Microalgae get the chance to be photoinhibited when light intensities that are somewhat higher than the light intensities at a specific growth rate top thereby causing generally reversible damage to the photosynthetic process [8]. Decreasing the possibility for photoinhibition. Maintaining a strategic distance from photoinhibition can increase the microalgal growth rate and biomass production [9]. Environmental components like temperature can be seen to affect microalgae growth rate, cell size, biochemical organization and nutrient requirement. The specific growth rate (μ) increases with increase in temperatures of microalgae species that are below their optimal temperatures but declines remarkably when temperature are increased above the microalgae species specific optimum temperature [10]. Minimal cell size are gotten when microalgae are grown at optimal temperatures [11] while non-optimal temperatures results in the decline of efficiency of carbon and nitrogen utilization [12].

2. Material and methods

2.1. Sample collection

The substrate used for this experiment are cassava peel and cassava waste water which were collected from cassava processing factory in Egberu-Ndoki area of Oyigbo L.G.A., Rivers state. An electric blender was used to blend the cassava peel into powdery form at a particulate size of 80/100 mesh after it has been washed and sundried. The microalgae stock culture were collected from pond water at African Regional Aquaculture Center (ARAC) in Aluu, Rivers state and enriched in a synthetic medium containing KNO_3 -0.132g, Na_2SiO_2 -0.066g, $\text{Na}_2(\text{PO}_4)_2$ -0.066g, EDTA-0.066g in one litre of water for 7 days [13].

2.2. Sample preparation

The Cassava peels were sun dried, ground into fine powder using a Panasonic electric blender, model (MX-J110P) to obtain a cassava peel with particle size of 80/100 mesh. Extracts were prepared by dissolving 10g of ground cassava peels in 100ml of distilled water as described by [14] and sterilized to destroy the pathogens and filtered using whatman's filter paper (No 1) while the cassava effluent were collected, sterilized and filtered.

2.3. Experimental studies

Cassava waste mixtures CP: CW at 160:40 and CW: CP at 160:40 were used throughout the duration of the experiment. temperature variations were monitored at 20 °C, 30 °C, and 35 °C labelled A-C at optical density of 670nm for 7 days. A positive control of *Chlorella vulgaris* grown on a novel synthetic medium and a negative control of the cassava waste mixture without inoculation labelled D and E was used to determine the effects of Temperature on *Chlorella vulgaris* cultivation from cassava waste mixture. Readings of the cell dry weight of *Chlorella vulgaris* was also taken for the 7 days. Photoperiod variations were monitored at 12:12, 6:18 and 18:6 labelled F-H at optical density of 670nm for 7 days. A positive control of *Chlorella vulgaris* grown on a novel synthetic medium and a negative control of the cassava waste mixture without inoculation labelled I and J respectively was used to determine the effects of Photoperiod on *Chlorella vulgaris* cultivation from cassava waste mixtures. Readings of the cell dry weight of *Chlorella vulgaris* was also taken for the 7 days.

2.4. Analyses

2.4.1. Optical Density

The Optical Density (OD) was determined using a spectrophotometer (Spectronic 721 model) set at 670nm. About 5ml of the growing culture were removed aseptically, placed in the cuvette after blanking and the absorbance was measured at 670nm.

2.4.2. Cell dry weight

The cell dry weight was determined from the methods of [15] to estimate the quantity of microalgal biomass produced. 5ml of the growing microalgal culture at different Temperature and Photoperiod levels were harvested by centrifugation at 3000rpm for 10 minutes. The cells were washed three times with physiological saline and dried at 50°C in a hot oven.

2.5. Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) was used to calculate the mean and Standard Deviation (SD). The Post hoc test (Scheffe and Duncan) was used to test for the significant difference at p-values ≤ 0.05 within the groups measured at 95% confidence level.

3. Results and discussion

The Temperature and Photoperiod variation on the growth and biomass production of *Chlorella vulgaris* on cassava waste are shown on fig. 1-8. The results showed that Temperature and Photoperiod variations significantly affected the growth and biomass production of the microalgae. The optimal Temperature was 30°C because it gave the highest absorbance and cell dry weight value while minimal Temperature was 20°C for absorbance and cell dry weight as shown on figures 1-4. The result revealed that growth and biomass production is enhanced when Temperature is within mesophilic temperature conditions than when it is at psychophilic conditions.

The optimal growth was obtained at photoperiod of 12:12 light:dark condition because it generated the highest growth when absorbance readings were taken as shown on figures 5 and 6 while optimum biomass was obtained at photoperiod of 16:8 light:dark conditions as it necessitated the production of the highest biomass as shown on figures 7-8.

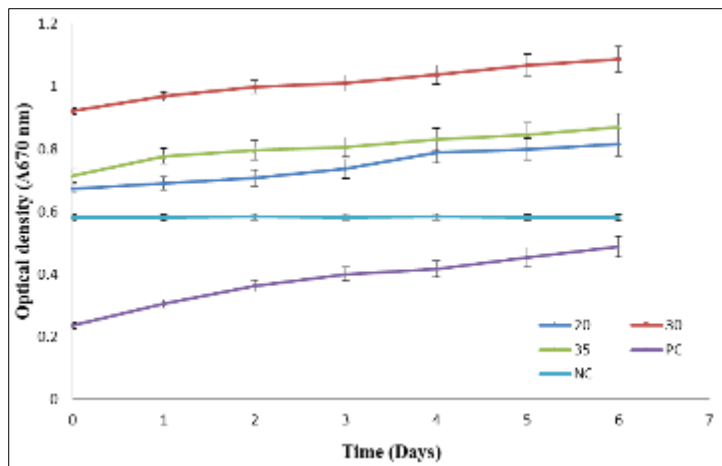


Figure 1 Changes in optical density with time of *Chlorella vulgaris* obtained from a cassava waste mixture at various temperature within the optimization period

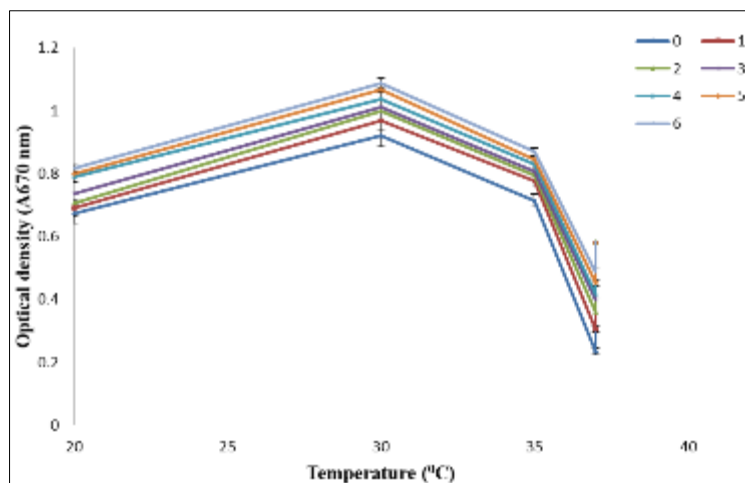


Figure 2 Changes in optical density of *Chlorella vulgaris* obtained at various temperature from cassava waste mixture during the optimization period

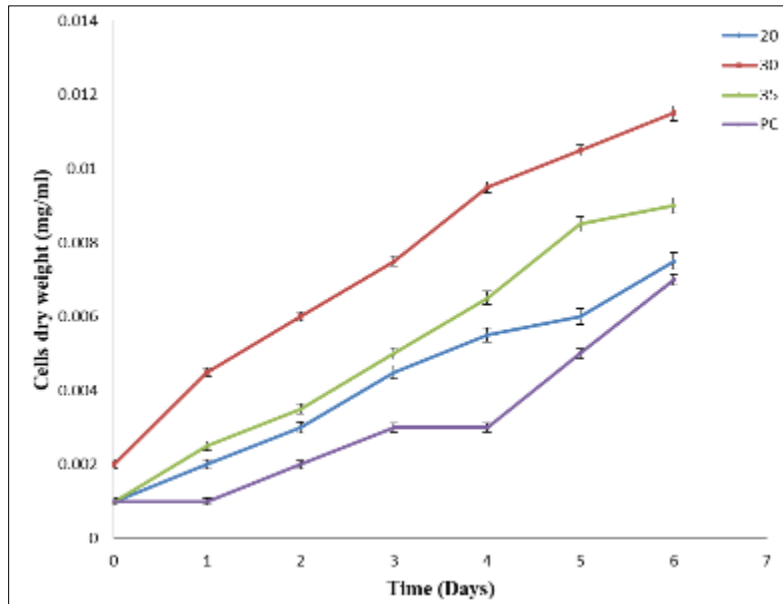


Figure 3 Changes in cells dry weight with time of *Chlorella vulgaris* obtained from a cassava waste mixture at various temperature during the optimization period

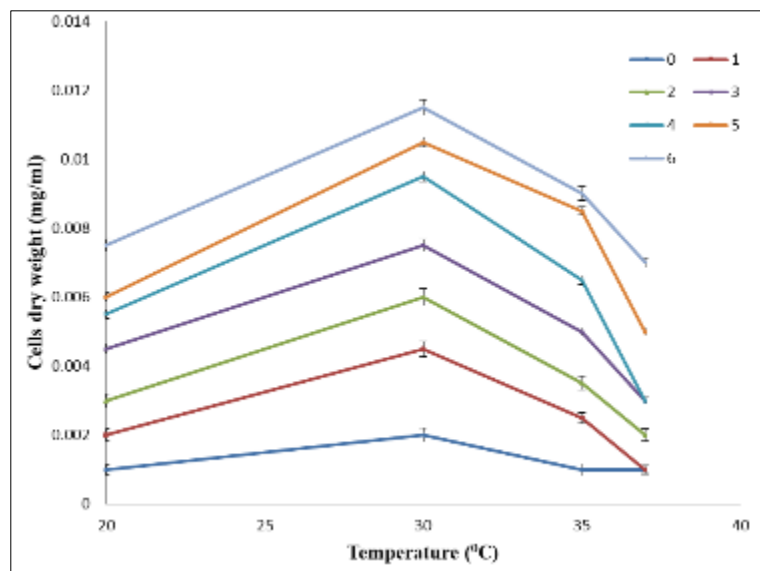


Figure 4 Changes in cells dry weight of *Chlorella vulgaris* at various temperature obtained from a cassava waste mixture during the optimization period

The growth response of the various Temperature conditions on *Chlorella vulgaris* cultivated on cassava waste is represented on fig. 1. It showed that thermophilic temperature conditions disrupted the growth of *Chlorella vulgaris* while at mesophilic Temperature, microalgal growth was enhanced. Microalgal response to Temperature showed that maximum growth was recorded at 30°C and minimum growth at 35°C for all the 7 days from the line graph on fig. 2. Biomass generation was at maximum at 30°C and minimum at Temperature of 20°C as shown on fig. 3 while the Temperature on biomass generation for the 7 days as represented on fig. 4. Maximum biomass was generated at Temperature of 30°C for the entire 7 days of biomass generation was monitored while the minimum biomass was generated at Temperature of 35°C as shown on fig. 4. This information is indicating that mesophilic conditions are most suitable for growth and biomass production of *Chlorella vulgaris* from cassava waste.

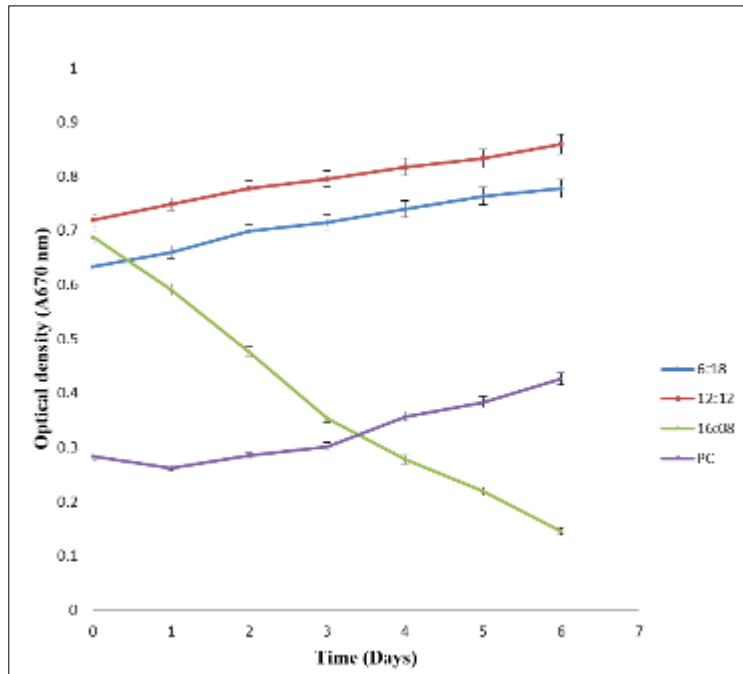


Figure 5 Changes in optical density with time of *Chlorella vulgaris* obtained from a cassava waste mixture at various photoperiod during the optimization period

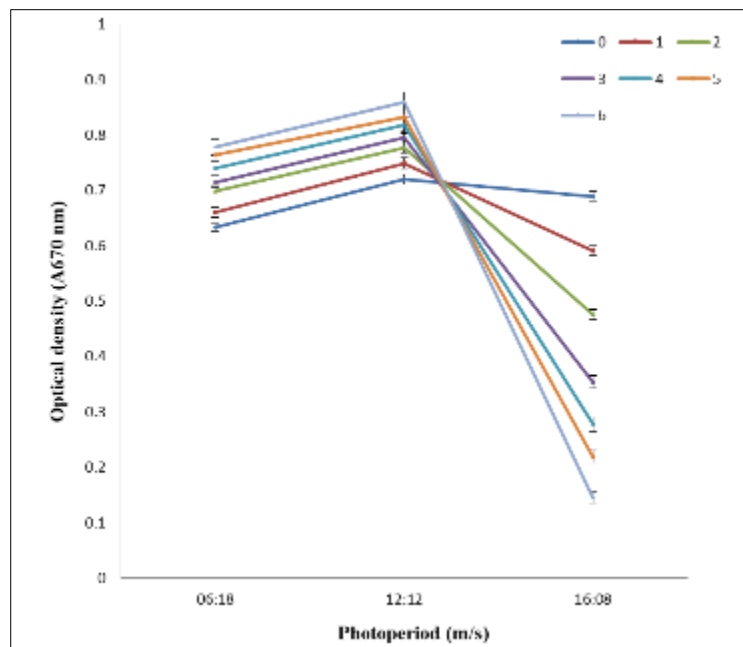


Figure 6 Changes in optical density of *Chlorella vulgaris* obtained at various photoperiod from a cassava waste mixture during the optimization period

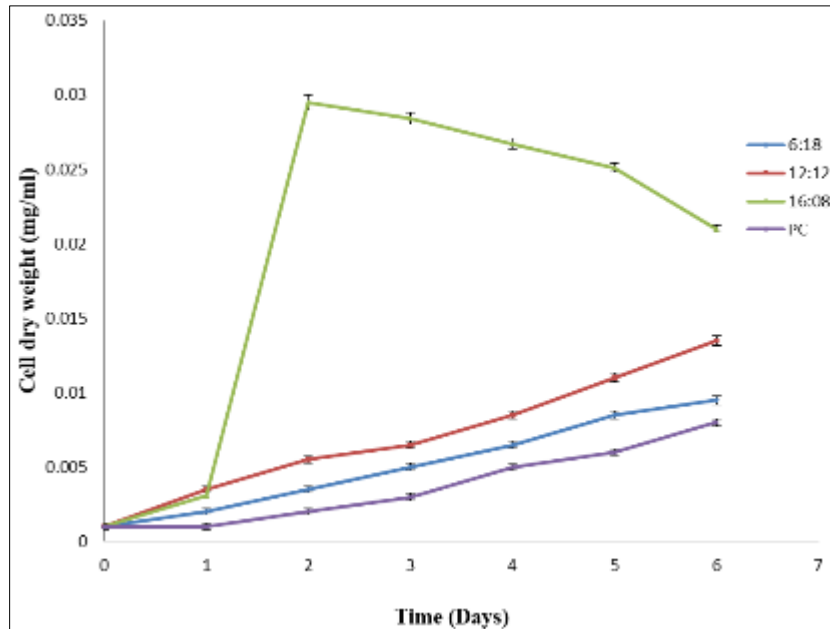


Figure 7 Changes in cells dry weight with time of *Chlorella vulgaris* obtained from cassava waste mixture at various photoperiod during the optimization

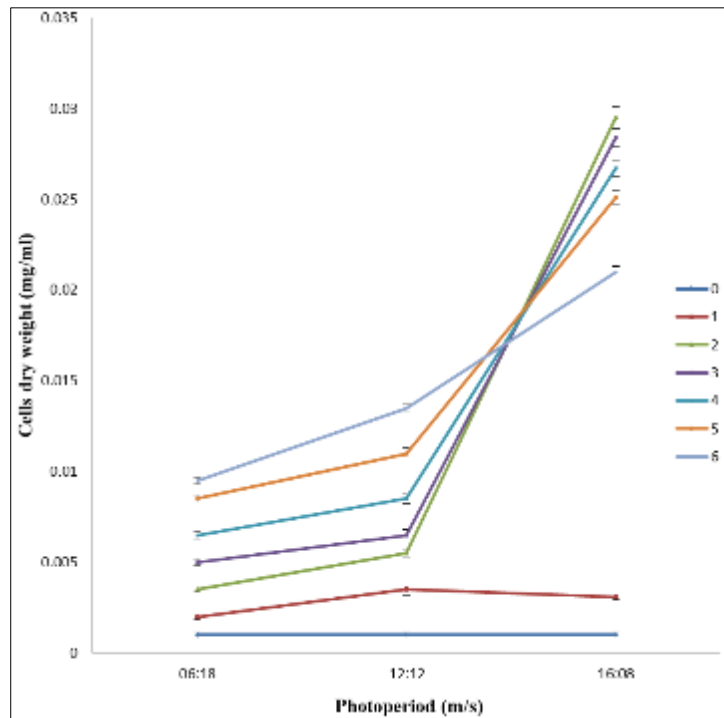


Figure 8 Changes in cells dry weight of *Chlorella vulgaris* obtained at various photoperiod from a cassava waste mixture during the optimization

The absorbance reading of the microalgal growth on different photoperiod showed that optimal growth were observed at photoperiod of 12:12 light:dark conditions while minimal growth were observed at photoperiod of 16:08 light:dark conditions as shown on fig. 5. The absorbance reading also showed that maximum growth for the various photoperiod were observed at the 7th day while the minimum growth were observed on day 0 as shown on fig. 6. This shows that microalgal growth increased with time over the 7 days period during which growth was monitored. Biomass production was maximum at photoperiod of 16:08 and minimum at photoperiod of 08:16 light:dark conditions as represented on

the line graph of fig.7. It was also observed that biomass generation was steady from day 0 and reached its peak on day 2 after which a steady decline was observed from day 3 to 6 as shown on fig 8.

4. Conclusion

From the results of this study, it is evident that variations in Temperature and Photoperiods significantly affected the growth and biomass production of *Chlorella vulgaris* in the mixture of cassava waste water and cassava peel water. It also reveals that mesophilic temperature conditions of 30°C are optimal for *Chlorella vulgaris* cultivation and biomass production on cassava waste mixture than psychrophilic and thermophilic temperature condition. The result also reported that photoperiods of 12:12 light:dark conditions were responsible for high microalgal growth while photoperiods of 16:08 light:dark conditions was responsible for high biomass generation.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors have declared that no conflict of interest exist in the work.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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