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Analysis of the optimum pH and salinity conditions for the cultivation and biomass production of *Chlorella vulgaris* from cassava waste

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Abstract

Biofuel serves as an alternative energy to the common fossil fuels currently in use globally and are drawing increasing attention worldwide as substitutes for petroleum-derived transportation fuels to help address challenges associated with petroleum derived fuels. Third generation biofuels, also termed advanced biofuels, are produced from fast growing microalgae and are potential replacements for conventional fuels. The growth and biomass production of these microalgae is dependent on the conditions they are cultivated such as pH and Salinity. Cassava waste mixtures were cultivated on *Chlorella vulgaris* stock culture at different concentration ratio at ambient temperature, natural light and dark conditions at 670nm absorbance for 14 days. Optimum growth was obtained at 160:40 for cassava peel water to cassava waste water CP:CW. pH variations 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 were checked to determine the optimum pH for the growth and biomass production of *Chlorella vulgaris* on the optimum cassava waste mixture concentration. It revealed that at pH 6.5, optimal growth and biomass production was achieved, minimal growth was observed at pH 8.0 while minimal biomass was produced at pH 9.0. Salinity variations of 5, 10, 15, 20, 25, 30, 35 and 40 mg/l were used to determine the growth response and biomass production of *Chlorella vulgaris.* It revealed that salinity variation at 10ppm will be necessary for highest growth on the cassava waste as well as in biomass production. The use of optimal pH and salinity can significantly increase biomass production thus enhancing biofuel production.

Keywords: Biofuel; *Chlorella vulgaris*; Biomass; pH; Salinity; Cassava waste

1. Introduction

Nigeria is the largest producer of cassava roots being responsible for about 20% of the total cassava product thus contributing its quota to the expansion of world cassava production from 200 million in 2004 to 240 million in 2009 (1). However, if the biofuel industry is to expand in future, researchers have recognized that non-food resources should be used so that the biofuel industries will not compete with the food market in terms of land for cultivation (2). Due to major threats to the environment by cassava processing industries as a result of improper cassava waste handling, an increasing future demands for cassava especially in Nigeria, may become a problem. Cassava waste products contain essential elements such as moisture 0.82%, Ash 2.71%, crude fibre 4.40%, crude protein 2.69%, crude lipid 3.92% and total carbohydrate 85.46 in addition to essential ions like nitrate, sulphate and phosphorus which can be used to grow *Chlorella vulgaris* for use in biofuel production (3).

Microalgae represent one-third (1/3) of the world's plant biomass and its renewable energy potential can be more environmentally sustainable, cost effective and more profitable if combined with processes such as waste management and utilization (4). These microalgae in turn are used to produce various beneficial biochemicals used in food, aquaculture, poultry and pharmaceutical industries (5). Biomass energy of microalgae is one of the potential renewable energy with the advantages of rapid reproduction, simple pyrolysis process and no environmental pollution (6).

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Microalgae biomass are important in anaerobic digestion during anaerobic biotransformation because it has high lipids, sugar and proteins content, and it does not contain recalcitrant lignin (7).The potential of *Chlorella vulgaris* to produce 37% dry weight of starch makes it to be considered as a rising feedstock for bioethanol production (8). Methane production of microalgal biomass through anaerobic digestion has been examined at different temperatures, pretreatments and co-substrates with few reviews having detailed the utilization of the complex substrates like cellulose (9), domestic waste water (10), paper recycling waste water (11) and solid animal manure (12) for methane production. The production of biomass energy from microalgal feed stock following bioremediation of waste water also enhance the material cost of biofuel production (13). 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 activities are solidly associated with biotic and abiotic conditions including: the availability of daylight; CO levels; pH; temperature and accessible nutrients.The biochemical composition of the microalgal cells are adverselyaffected by environmental conditions such as light, and temperature, the availability of non-mineral nutrients, macronutrients, and micronutrients, (14). The growth rate of microalgae will be fundamentally expanded at suitable conditions to generate more biomass (15). With the increase of pH, CO_2 in water is converted to HCO_3^-which is the mainly existing formation of carbon in weak alkaline and is majorly utilized by microalgae. Salinity is another important factor that alters the biochemical composition of algal cells (salinity refers primarily to sodium chloride concentration unless otherwise specified). Exposing algae to lower or higher salinity levels than their natural (or adapted) levels can change growth rate and alter composition. Generally, seawater microalgae can tolerate higher salinity rather than fresh water microalgae. Microalgae have its own system to adjust salinity range and studies have shown that microalgae have its own optimal growth salinity, thus salinity levels which are higher or lower than the optimal level will be harmful to algal growing rate.

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, $Na_2(PO_4)_2$ -0.066g, EDTA -0.066g in one litre of water for 7 days (16).

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 (17) 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. pH variations were monitored at 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0 labelled A-G at optical density of 670nm for 7days by adjusting the pH using 1.0M NaOH and 1.0M H_2SO_4. A positive control of *Chlorella vulgaris* grown on a novel synthetic medium and a negative control of the cassava waste mixture without inoculation labelled H and I was used to determine the effects of pH on *Chlorella vulgaris* cultivation from cassava waste mixture. Readings of the cell dry weight of *Chlorella vulgaris* was also taken for the 7days. Salinity variations were monitored at 5, 10, 15, 20, 25, 30, 35 and 40mg/l abelled J-Q at optical density of 670nm for 7days. A positive control of *Chlorella vulgaris* grown on a novel synthetic medium and a negative control of the cassava waste mixture without inoculation labelled R and S respectively was used to determine the effects of salinity on *Chlorella vulgaris* cultivation from cassava waste mixtures. Readings of the cell dry weight of *Chlorella vulgaris* was also taken for the 7days.

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.

2.5. Cell dry weight

The cell dry weight was determined from the methods of (18) to estimate the quantity of microalgal biomass produced. 5ml of the growing microalgal culture at different pH and salinity levels were harvested by centrifugation at 3000rpm for 10 minutes. the cells were washed three times with physiological saline and dried at 50^0C in a hot oven.

2.6. 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 pH and salinity variation on the growth and biomass production of *Chlorella vulgaris* on cassava waste are shown on fig. 1-8. The results showed that pH and salinity variations significantly affected the growth and biomass production of the microalgae. The optimal pH was 6.5 because it gave the highest absorbance and cell dry weight value while minimal pH was 8.5 for absorbance and pH 8.0 for biomass production as shown on figures 1-4. The result revealed that growth and biomass production is enhanced when pH is close to neutral conditions than when it is alkaline.

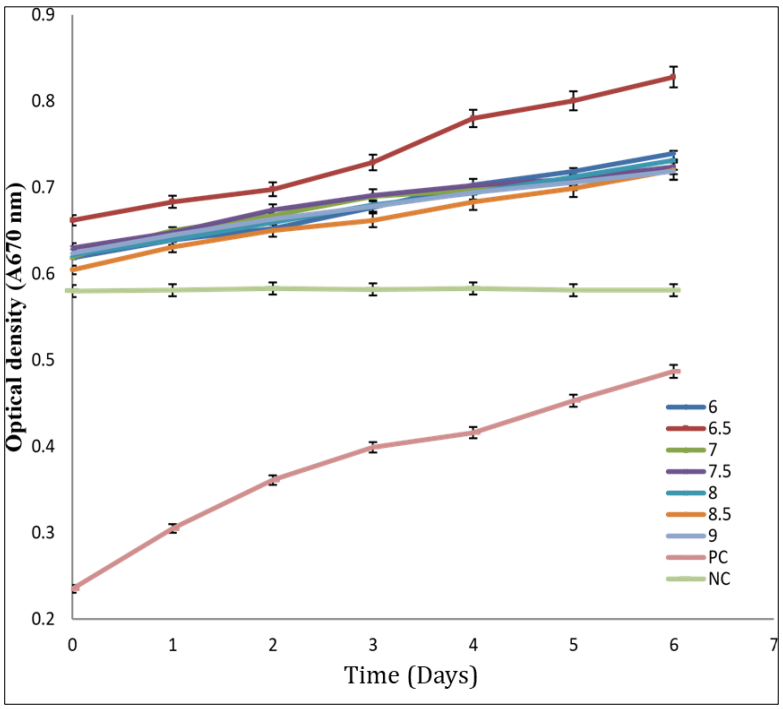

Figure 1 Changes in optical density with time of *Chlorella vulgaris* obtained from a cassava waste mixture at various pH during the optimization period

The optimal salinity condition was obtained at the salinity of 10mg/ml because it generated the highest growth when absorbance readings were taken and also necessitated the production of the highest biomass as shown on figures 5-8. The continuous increase in salinity of the cassava waste medium resulted in constant growth decline and biomass generation. This is because the microalgae could not respond positively to the different salt stress above 10mg/ml.

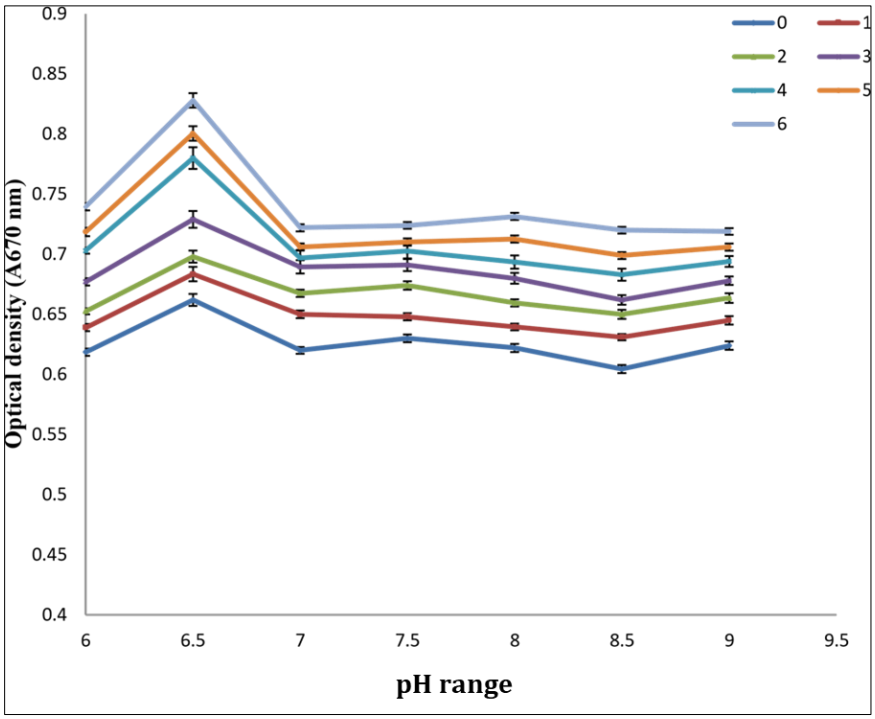

Figure 2 Changes in optical density of *Chlorella vulgaris* obtained at various pH from a cassava waste mixture during the optimization period

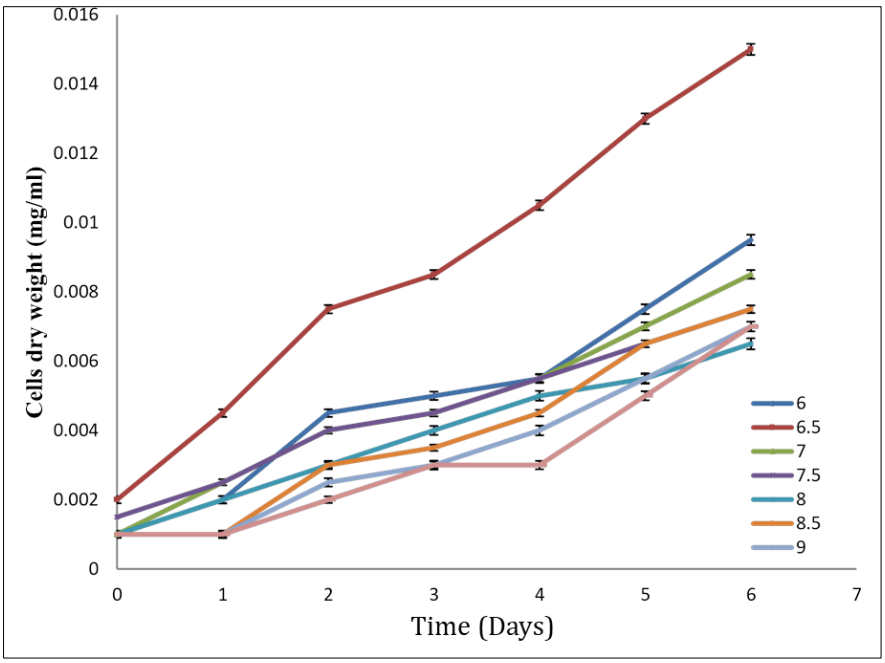

Figure 3 Changes in cells dry weight with time of *Chlorella vulgaris* obtained from a cassava waste mixture at various pH during the optimization period

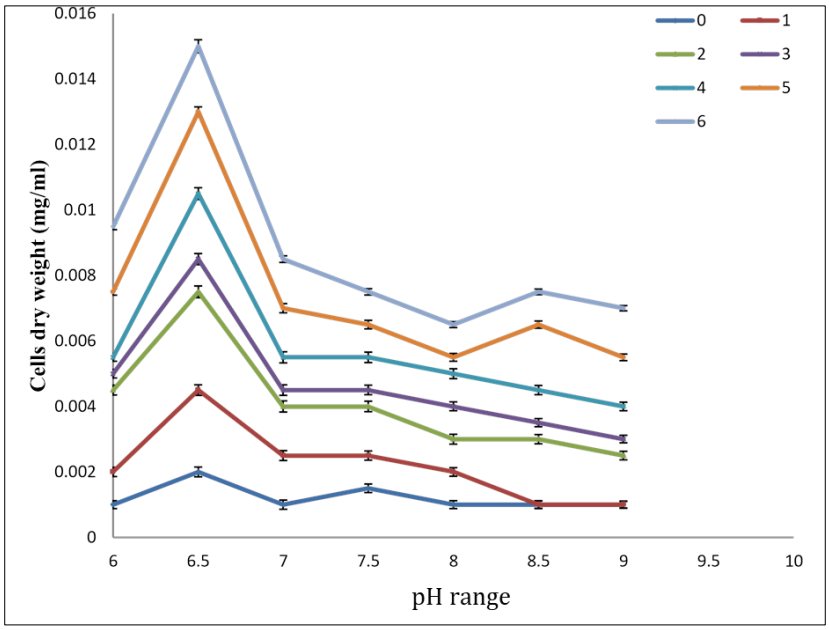

Figure 4 Changes in cells dry weight of *Chlorella vulgaris* obtained at various pH from a cassava waste mixture during the optimization period

The growth response of the various pH conditions on *Chlorella vulgaris* cultivated on cassava waste is represented on fig. 1. It showed that alkaline pH disrupted the growth of *Chlorella vulgaris* while at pH close to neutrality, microalgal growth was enhanced. Microalgal response to pH showed that maximum growth was recorded at pH 6.5 and minimum growth at pH 8.5 for all the 7 days from the line graph on fig. 2. Biomass generation was at maximum at pH 6.5 and minimum at pH 8.0 as shown on fig. 3 while the pH on biomass generation for the 7 days is as represented on fig. 4. Maximum biomass was generated at pH 6.5 for the entire 7 days of biomass generation was monitored while the minimum biomass was generated at pH 7.0 at day 0, pH 8.5 and 9.0 for day 1, pH 9.0 for day 2, 3 and 4, pH 8.0 for day 5 and 6 as shown on fig. 4. This information is indicating that alkaline conditions are not suitable for growth and biomass production of *Chlorella vulgaris* from cassava waste.

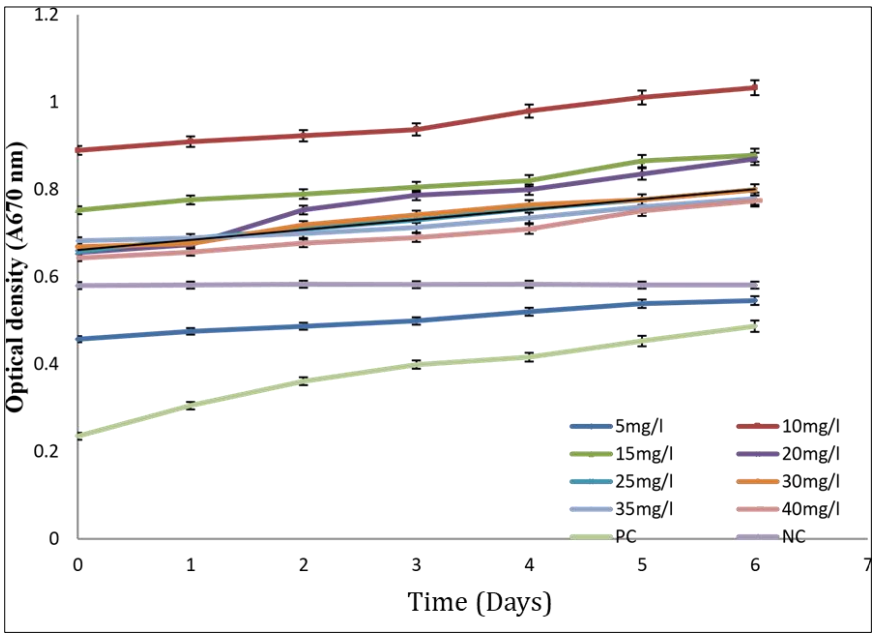

Figure 5 Changes in optical density with time of *Chlorella vulgaris* obtained from a cassava waste mixture at various salinity concentrations during the optimization period

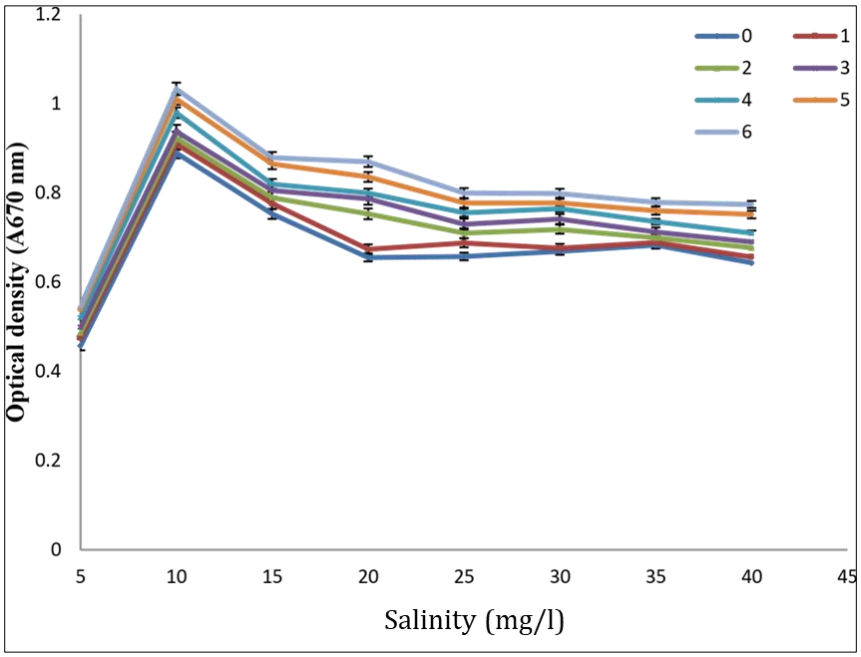

Figure 6 Changes in optical density of *Chlorella vulgaris* obtained at various salinity concentrations from a cassava waste mixture during the optimization period

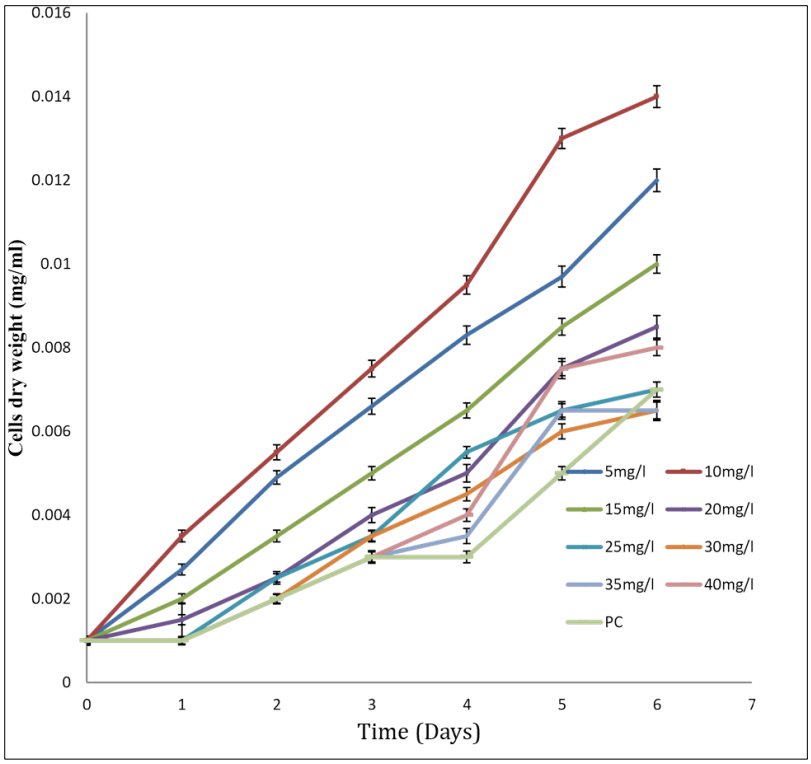

Figure 7 Changes in cells dry weight with time of *Chlorella vulgaris* obtained from a cassava waste mixture with various salinity concentrations during the optimization period

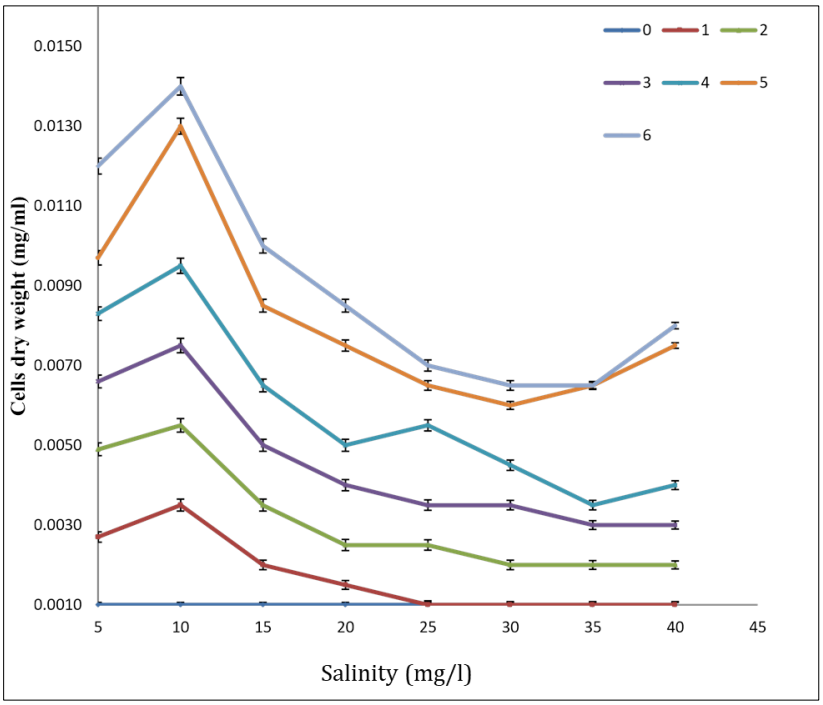

Figure 8 Changes in cells dry weight of *Chlorella vulgaris* obtained at various salinity concentrations from a cassava waste mixture during the optimization period

The absorbance reading of the microalgal growth on different salinity concentration showed that optimal growth were observed at salinity concentration of 10mg/ml while minimal growth were observed at salinity concentration of 5mg/ml as shown on fig. 5. The absorbance reading also showed that maximum growth for the various salinity concentration were observed at the 6th 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 salinity of 10mg/ml and minimum at salinity of 30mg/ml and 35mg/ml as represent on the line graph of fig. 7. It was also observed that biomass generated at salinity level of 40mg/ml was higher than salinity level of 25, 30 and 35mg/ml. This might be that the microalgae is a halophile. Biomass production was on a steady increase from day 0 and the 6th day accounted for the maximum biomass production as shown on fig. 8.

4. Conclusion

From the results of this study, it is evident that variations in pH and Salinity 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 near neutral conditions of pH 6.5 are optimal for *Chlorella vulgaris* cultivation and biomass production on cassava waste mixture than alkaline pH condition.

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.

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