Original Research Article

Journal of Biological Research & Biotechnology

Bio-Research Vol. 21 No.2; pp. 2058-2064 (2023). ISSN (print):1596-7409; eISSN (online):2705-3822

Outdoor cultivation of microalgae in a medium enriched with poultry droppings for biomass production

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Abstract

Microalgae require nutrients and optimal pH for good growth and metabolite production. Microalgae biomass has various applications in food and feed, pharmaceuticals, bio-energy, aquaculture, cosmetics and other related industries. However, the cost of media represents a significant percentage of the total production cost. The aim of this work was therefore, to develop a cheap medium for efficient outdoor cultivation of microalgae and to find the optimum pH for biomass production. Three *Chlorella* species designated A, B and C were isolated from three stagnant water bodies at Amoke Lane, University market road in Nsukka town by using BG-11 enriched with poultry droppings. Poultry droppings medium was prepared by drying under the sunlight for 5 days. The dried poultry droppings were crushed into a fine powder and 200 g was weighed and soaked in 1000 ml of distilled water for 3 days and filtered using a muslin cloth. A mixture of the poultry droppings medium and the conventional BG-11 medium was prepared by mixing equal volume of each medium. The growth and biomass productivity of the three local isolates in BG-11 medium were compared with those in poultry medium, and a mixture of poultry and BG-11 media. The results showed that the growth of the three isolates in poultry medium alone were significantly higher than the values obtained in BG-11 and a mixture of BG-11 and poultry medium ($p< 0.05$). With poultry medium, the maximum optical densities of the cultures were 2.10± 0.07, 2.56± 0.09 and 3.35± 0.11 for isolates A, B, and C respectively. However, with a mixture of BG-11 and poultry medium, the maximum optical densities were 0.83± 0.01, 1.67± 0.05 and 0.57± 0.02 respectively. BG-11 supported the least growth of the three isolates, giving maximum optical densities of 0.38± 0.01, 0.42± 0.03 and 0.52± 0.02 respectively. All the three isolates had their best growth in poultry medium at pH of 5.0. The results show that poultry medium, which is cheap and can be easily prepared from poultry droppings, is very good for cultivation of some species of microalgae.

Keywords: Algae biomass, poultry droppings, BG- 11 nutrient, outdoor cultivation and pH

Received February 23,2023; **Revised** August 1, 2023; **Accepted** August 4, 2023

https://dx.doi.org/10.4314/br.v21i2.10
Journal Homepage: http://www.bioresearch.com.ng.
Publisher*: Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.*

INTRODUCTION

Microalgae are unicellular photosynthetic microorganisms living in water environments, which convert sunlight, water, carbon dioxide and other nutrients to algae biomass (Khan *et al*., 2018). For hundreds of years, green microalgae biomass has been used as nutritional supplement and sources of many other useful metabolites. Microalgae biomass has recently attracted considerable interest worldwide, due to their extensive application potential in the renewable energy, biopharmaceutical, aquaculture, cosmetics and nutraceutical industries (Khan *et al*., 2018). Biomass of several microalgae species have been investigated for their potential as value-added products with remarkable pharmacological and biological qualities (Khan *et al*., 2018; Lam and Lee, 2012; Andrew *et al*., 2008; Nigam and Singh, 2011; Varma and Sharma, 2018; Clarens *et al*., 2010; Christenson and Sims, 2011; Harun *et al*., 2010; Gendy and El-Temtamy, 2013; Mata *et al*., 2010).

Algae biomass is rich source of protein, enzymes and minerals such as vitamins A, C, B1, B2 and niacin etc. Thus, it is presently used as a major source of food throughout the world and especially in Asian countries such as China, Japan and Korea. In health and pharmaceutical; medicines, vitamins, vaccines, nutraceuticals and other nutrients which when made using animals or plant are very expensive, can be produced using microalgae biomass (Khan *et al*., 2018; Michael, 2013; Pulz and Gross, 2004).Microalgae biomass is used in cosmetics and has the capacity to detoxify, cleans and tones the skin. The biomass has natural anti-cellulite and anti-aging properties. It also helps in increasing the elasticity and suppleness of the skin (Yarkent *et al*., 2020). Microalgae biomass is used in aquaculture. Shrimps and some finish cultures have an essential requirement for microalgae in hatchery and nursery. Fishmeal is the preferred protein ingredient of feed in aquaculture industry. Microalgae are used worldwide as alternate protein sources replacing fishmeal successfully (Roy and Pal, 2014). Microalgae growth and chemical composition are mainly controlled by light, available carbon dioxide, pH, and nutrients. Other factors, such as salinity, can be of vital importance to some species. Growth medium must provide sufficient nutrients for microalgae growth (Radmann, 2008; Plaza *et al*., 2009). The cost of media represents a significant percentage of the total production cost. Conventional BG-11 (blue-green algae medium) has been used for cultivation of many species of microalgae. However, BG-11 is very complex and costly, while many of the components are not easily available in some of the developing counties such as Nigeria. Natalia and Igor (2021) reported microalgae cultivation in poultry waste for biodiesel production using photoreactor. As it stands now there is no report on outdoor cultivation of microalgae in medium enriched with poultry droppings for biomass production. Development and utilization of wastes such as poultry droppings under outdoor cultivation will help to reduce the cost of cultivation of microalgae. Therefore, the aim of this work was to develop a cheap medium for efficient outdoor cultivation of microalgae and to find the optimum pH for biomass production.

MATERIALS AND METHODS

Preparation of Blue-Green (BG) 11 medium

BG 11 growth medium was composed of (per litre): 0.25 g $NaNO_3$; 0.04 g K_2HPO_4; 0.075 g $MgSO_4 \cdot 7H_2O$; 0.027 g $CaCl_2 \cdot 2H_2O$; 0.006 g $C_6H_8O_7$ 0.006 g $C_6H_8O_7$.nFe.nNH_3; 0.02 g $NaCO_3$ for stock solution 1. The stock solution 2 was prepared by dissolving (per litre): 2.860 g H_3BO_3; 0.222 g $ZnSO_4 \cdot 7H_2O$; 1.81 g $MnCl_2.4H_20$; 0.079 g $CuSO_4 \cdot 5H_2O$; 0.390 g $Na_2MoO_4 \cdot 2H_2O$; and 0.0494 g Co $(NO_3)_2.6H_20$. BG 11 Media was prepared by adding 1ml of Stock 2 into 1000 ml of stock 1 solution.

Preparation of poultry droppings medium

Poultry droppings were collected from Opi poultry farm at Nsukka in Enugu state. Foreign objects were removed from the sample and the poultry droppings were dried under the sunlight for 5 days to obtain constant weight. The dried poultry dropping was crushed into a fine powder and 200g was soaked in 1000ml of distilled water for 3 days. The mixture was filtered using a muslin cloth and autoclaved at 121^o C for 15 minutes.

Microalgae Isolation

Water sample was collected from three stagnant water bodies at Amoke Lane, University market road, Nsukka in Enugu State, Nigeria. About 100 ml of Blue-Green (BG) 11 medium was mixed with 50 ml of poultry droppings liquid medium,

thereafter, 50 ml of the water sample was added and allowed to grow outdoor for 10 days under sunlight. The cultures were subsequently sub-cultured and purified by inoculating 20 ml of the culture in 250ml conical flask containing 100 ml of BG 11 and 100 ml of poultry droppings liquid medium.

Identification

The microalgae species were identified under the light microscope based on morphological features observed and comparison with online microalgae atlas. The identified microalgae were sub-cultured in 200ml fresh Blue-Green 11 medium in 500ml flask and under Sunlight. Sub-culturing was done every 10 days.

Cultivation of the microalgae with different media

About 200 ml of either, BG 11, poultry dropping or a mixture of BG 11 and poultry droppings in equal ratio was dispensed in different 250 ml conical flasks. Each flask was inoculated with 20 ml of 10 days old microalgae inoculum. The conical flasks were covered with cotton wool and incubated outdoor under sunlight for 12 days.

Effect of pH on biomass production

The effect of the following pH (3, 4, 5, 6, 7 and 8) on biomass production was carried out. These were done by adjusting the pH of the poultry droppings medium with 1N HCl or 1N NaOH. Thereafter, 20 ml of the microalgae isolates was inoculated in 200 ml of the poultry dropping medium and incubated outdoor under the light for 12 days.

Biomass measurement

During the cultivation, samples were taken at 2 day intervals and the biomass concentrations were estimated by measuring the optical densities (OD) at 620 nm, using a Spectrophotometer 722S B. Bran Scientific and Instrument Company, England.

Statistical analysis

Data obtained were subjected to one- way analysis of variance (ANOVA) and the means were separated using the least significant difference.

RESULTS AND DISCUSSION

Cultivation of microalgae species A, B and C in blue green (BG) 11 and poultry droppings media

The three isolates of microalgae were identified as *Chlorella* species and were designated isolate A, B, and C. Figure 1 shows cultivation of microalgae isolate A in 200 ml of BG-11 medium, a mixture of 100 ml BG-11 medium and 100 ml poultry droppings medium and 200 ml poultry droppings alone. Isolate had the highest growth in poultry dropping medium, giving an optical density of 2.5± 0.08. This was followed by BG-11 medium mixed with poultry dropping with optical density of 1.19± 0.02 while the least growth was observed with BG-11 medium alone (OD = 0.38± 0.01). With poultry medium, the maximum OD was achieved on the 3rd day, thereafter there was a decline in the OD value. This is in agreement with Venckus *et al*. (2017) who reported the same growth trend during cultivation of green algae *Chlorella vulgaris* cultivation in municipal wastewater. Arumugam *et al*. (2020) reported the same trend of algae growth during enhancement of targeted microalgae species growth using aquaculture sludge extracts. They reported that natural growth-promising nutrients extracted aquaculture sludge waste can be used to maximize microalgae growth. These results with the present research suggested that organic nutrients support better growth of microalgae than inorganic nutrients. A poultry dropping is an organic nutrient while BG-11 is an inorganic nutrient. The declined growth in the entire medium after attending its maximum biomass might be attributed to depletion in nutrient and accumulation of some inhibitory compounds.

Fig 2 shows the growth of isolate B in the three media. Isolate B had the highest growth ion poultry dropping medium, giving an OD of 2.56± 0.09 while the OD in the mixture of BG 11 and poultry dropping was 1.67± 0.05. The least OD of

0.49± 0.02 was obtained in BG 11 alone. In all the media, the maximum OD of isolate B attained on day 2, after which there were decreases in the OD values. This is in agreement with Praba *et al*. (2016) who reported the same pattern of growth and cell count during their study on the effect of different culture media on the growth and oil yield in selected marine microalgae. Llavarasi *et al*. (2011) also reported that the maximum cell concentration was attained on day 2 in some of their results during optimization of various growth media for growth of freshwater microalgae. This result with the present work suggested that different microalgae species vary in their growth rates. Isolate B is a very fast-growing algae when compared to other isolates.

In comparison with other isolates, the growth of isolate C in poultry droppings was the highest, reaching OD of 3.35± 0.11 on day 2. The OD attained in BG11 and a mixture of BG11 and poultry droppings were 0.52± 0.02 and 0.95± 0.01 respectively (fig 3). This is in agreement with Praba *et al*. (2016) who reported the same pattern of growth and cell count during their study on the effect of different culture media on the growth and oil yield in selected marine microalgae. Llavarasi *et al*. (2011) reported that the maximum cell concentrations were attained day 2 and day 3 during optimization of various growth media for freshwater microalgae.

Effect of pH on outdoor cultivation of microalgae isolate A, B and C in poultry droppings medium

Table 1 shows the optical density (O.D) of the C*hlorella* species isolates at different pH values during outdoor cultivation in poultry medium. As the pH increased, the optical density increased but the optimum pH for the maximum biomass production was pH 5. At this pH value, the optical densities of isolates A, B and C were 3.81± 0.01, 3.51± 0.05 and 4.13± 0.06 respectively. This result is in agreement with Gong *et al*. (2014) who reported that light and pH are important parameters for microalgae cultivation. Qiu *et al*. (2017) reported pH 6 as the optimum for cell growth during their research on the effects of pH on cell growth, lipid production and carbon (iv) oxide fixation by *Chlorella sorokiniana*. They found that the pH optimum for growth of *Chlorella* sp is close to neutrality. This result with our findings, suggest that the different *Chlorella* species have different pH requirement for their maximum growth. Sarker and Salam (2019) reported the possibility of improving the process performance by controlling pH during indoor and outdoor cultivation of *Chlorella vulgaris* and its application in wastewater treatment.

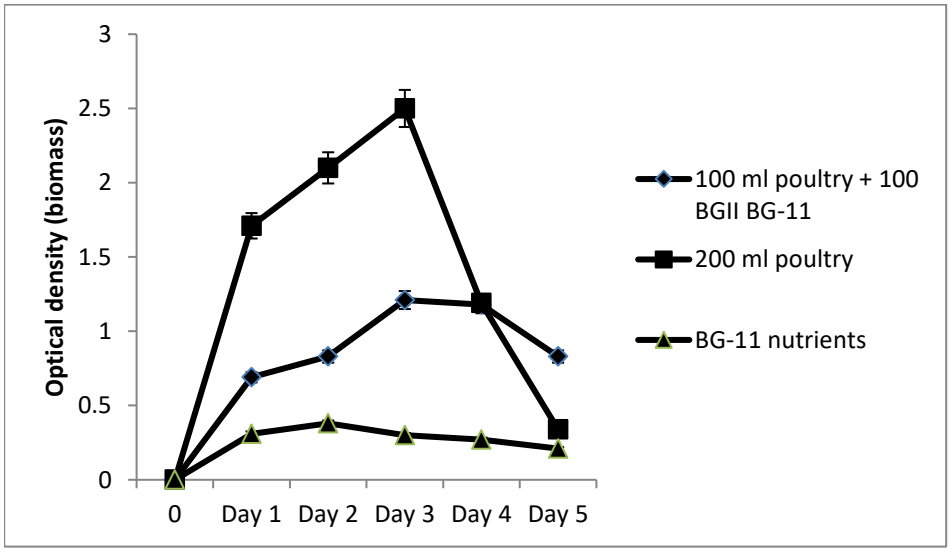

Fig. 1; Outdoor Cultivation of microalgae isolate (A) in BG 11, poultry droppings and a mixture of BG 11 and poultry dropping media

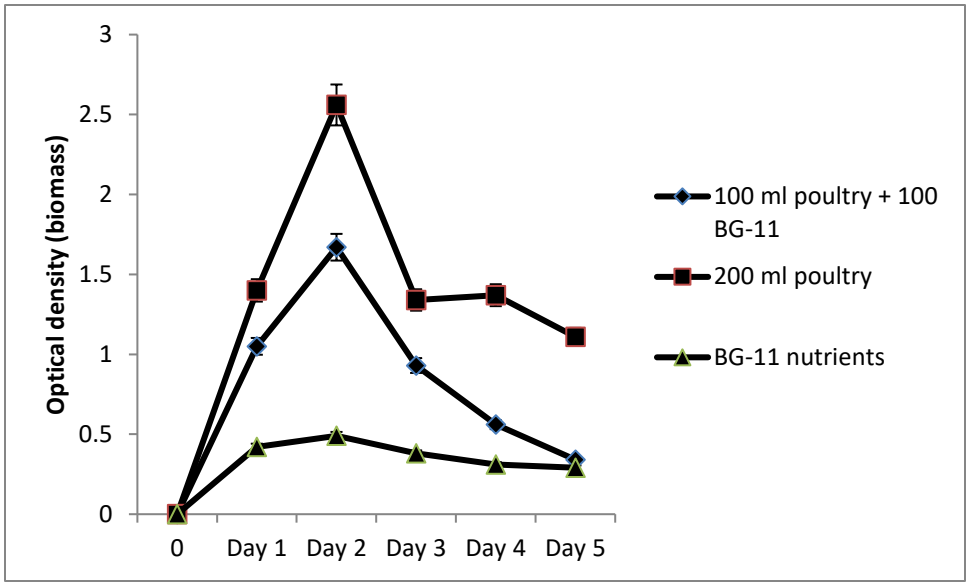

Fig. 2; Outdoor Cultivation of isolate (B) in BG 11, poultry dropping and a mixture of BG11 and poultry droppings media.

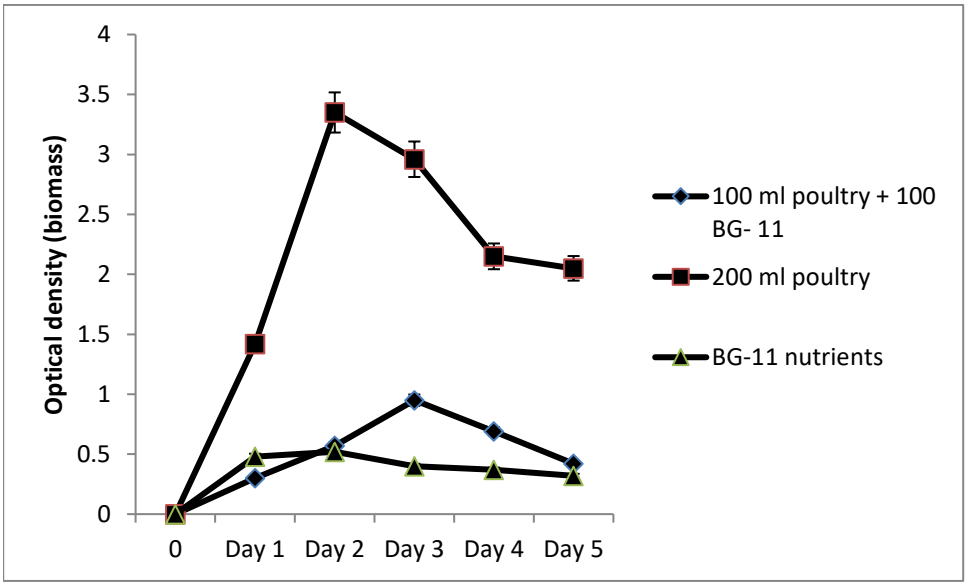

Fig. 3; Outdoor Cultivation of isolate (C) in BG 11, poultry dropping and a mixture of BG11 and poultry dropping media

Table 1 Effect of pH on outdoor cultivation of microalgae isolates in a poultry droppings medium

pH	Isolate A (O.D)	Isolate B (O.D)	Isolate C (O.D)
3	1.081± 0.02	1.054 ± 0.01	1.271 ± 0.02
4	2.193 ± 0.04	2.035 ± 0.02	2.473 ± 0.04
5	3.809 ± 0.01	3.504 ± 0.05	4.134 ± 0.06
6	3.262 ± 0.05	3.014 ± 0.04	3.479 ± 0.07
7	2.712 ± 0.02	2.861 ± 0.03	2.921 ± 0.08
8	1.603 ± 0.01	1.163 ± 0.01	1.381 ± 0.02

CONCLUSION

This study has revealed that poultry droppings support higher growth of microalgae than the conventional BG-11 medium. It is recommended that poultry droppings should be harnessed for biomass production. A poultry dropping is a rich waste material and it is sustainable, economical and can easily be produced for large scale cultures. This research work recommends adequate monitoring and control of pH for the maximum biomass production. More effort should be put in place to purify the poultry droppings for proper cultivation of microalgae biomass under outdoor conditions.

Conflict of interest

There is no conflict of interest

Acknowledgement

Authors are thankful to ESUT management for the space opportunity and significant contribution to the success of this work. There was no specific funding received for this research.

Authors' contributions

EIB conducted the experiment and drafted the manuscript. OJC supervised the study and proofread the manuscript. Both authors read and approved the final draft of the manuscript

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