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Effect of Growth Media and pH on Microalgal Biomass of *Chlorella vulgaris* for Biodiesel Production

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ABSTRACT

The increased industrialization and overuse of natural resources for energy, such as fossil fuels, have led to the energy crises and environmental issues that plague the world in the twenty-first century. The production of biodiesel from algae has recently gained attention as a potentially useful alternative fuel that is both environmentally friendly and easy to obtain. In this work, the effects of various pH levels on algae biomass and oil production from *Chlorella Vulgaris* were studied. The growth of the biomass concentration was monitored using a spectrophotometer. The biomass of *C. vulgaris* obtained from the test was subjected to oil extraction using the chemical solvent method. Out of the five media compositions tested (MBG-11, BG-11, BBM, M8 and N8), MBG-11 recorded the highest biomass concentration at pH 8 (0.6 mg/L/D) and N8 recorded the least biomass concentration at pH 6 (0.49 mg/L/D). The highest percentage of oil was extracted from the *C. vulgaris* in BBM at pH 6 (31.22%) while the lowest oil was recorded in M8 at pH 8 (14.75%). In conclusion, the best medium for *C. vulgaris* biomass production was MBG-11 medium while the best medium for oil Production from this microalga was Bold Basal Medium (BBM).

INTRODUCTION

Extracting biodiesel from microalgae is a renewable energy source that is gentle on the environment. The primary difficulties in producing algal biodiesel [1] are related to optimizing biomass and lipid productivities. Whether it's from microalgal lipids, vegetable oils, or animal fats, biodiesel is made up of mono-alkyl esterstriglycerides. Transesterification is a process used to reduce the viscosity of oils and fats that are not compatible with transportation vehicle engines [2]. Biodiesel can be used as a supplement to or replacement for regular diesel because its main characteristics are similar to and compatible with those of regular diesel. To replace fossil fuels, biodiesel made from oil crops could be used as it produces no greenhouse gases. Sadly, the current demand for transportation fuels cannot be met by biodiesel produced from oil crops and animal fat [3]. Microalgae are photosynthetic organisms that can serve as a feedstock for a wide variety of products, including medicines, cosmetics, toothpaste, alginates, agar, and renewable fuels like biodiesel, biohydrogen, and bioethanol [4, 5]. Because they reduce wasteful use of natural resources like water and land, they are becoming increasingly popular [6]. You can use them as biofertilizers and bioremediators [7].

As a eukaryotic (unicellular) chlorophyte, *Chlorella vulgaris* is a type of plant with cells. Their low nutrient needs make them ideal for commercial cultivation, and they are already being put to use in a wide range of products like protein-rich dietary supplements, pharmaceutical chemicals, and colorants (used in food and cosmetics). Additionally, they mature much more rapidly than plants or many strains of microalgae, and their oil content is much higher, making them excellent candidates for biodiesel production. *C. vulgaris* has long been cultured as a model strain for Biofuel production in the United States and Europe [6], and one strain has shown great promise as a source of oil [9]. This work investigated the effects of various pH levels on algal biomass and oil production from *Chlorella vulgaris.*

MATERIALS AND METHODS

Sample and Sampling site

The algae sample was previously isolated in 2021 from an open pond at Hauren shanu along Bayero University Kano Road, Gwale Local Government Kano State, Nigeria (unpublished data).

Preparation of Nutrient Media and pH

The four best nutrient growth media that support the growths of *Chlorella vulgaris* as reported by *Wang et al.* [10] research were used. Bold Basal Media (BBM) [11], Modified Blue Green Media (BG-11) [12], N-8, M-8 [13] and BG-11 [14], was added making five different growth media. All the five media were prepared using standard procedure and adjusted to the desired pH levels (6, 7 and 8) with diluted HCl (Hydrochloric acid) or NaOH (Sodium hydroxide) after every 24 h for 12 days.

Culturing of *Chlorella vulgaris*

C. vulgaris was cultured under three pH conditions of 6,7 and 8 in five different media i.e. BG-11, MBG-11, BBM, M8 and N8 in 500 mL Erlenmeyer flasks (conical flask) each containing 400 mL media. After obtaining a pure culture of the *C. vulgaris* on different agar plates, each plate was washed with a BBM medium under a safety cabinet and then aseptically transferred into 500 mL conical flask containing 400 mL of BBM growth medium and incubated at 20-25 $^{\circ}$C with aeration for 12 days.

The conical flask containing the culture was held within a shaker (New Brunswick Innova 4000 incubator shaker) to prevent sedimentation of microalgae. The shaker was set to shake at a rate of 150 rpm and adjusted its light/dark cycle, with 16/8hrs under continuous fluorescent lamp illumination (6000–6500 lx) [15].

In order to get rid of the excess media, the algae stock culture (grown in 400 mL BBM media for 12 days) was centrifuged at 1800 rpm for 30 min. To maintain a consistent algal concentration, the dried algae were resuspended in deionized water. All experiments followed the same protocol for preparation [13] unless otherwise specified.

Determination of Biomass Concentration

The cleaned stock culture from BBM growth media was transferred into twelve 500 mL conical flasks aseptically and incubated at 20 – 25 $^{\circ}$C for 24hr under a shaking condition of 150 rpm. The biomass concentration was obtained after each 24 h and in triplicate for 12 days using a UV visible spectrophotometer at an absorbance of 686 nm. The concentration was calculated on the linear equation obtained from the calibration curve, as y = mx + b, and also while the pH measurements were taken at time 0 – 12 days after every 24 h (±1 h) using 5 in 1 pen-type water quality meter (Model Number : EZ-9909).

Preparation of algae for oil extraction

Coagulant was obtained from the shells of boiled chicken eggs. Distilled water was used to wash the eggshells before they were dried at 35 degrees Celsius. The powder was made by mechanically sieving dried eggshells through a 325 mesh sieve to obtain a fine consistency. For 30 min, the eggshell powder (20 mg) was stirred constantly in 2 mL of 0.1 mol/L acid solutions. Finally, 20 mL of deionized water was added to the acid solution, bringing the total eggshell concentration down to 1000 mg/L [16].

The eggshell solution (1000 mg/L) was added to each algal sample to coagulate the algal cells from the media and centrifuged at 5000 rpm for 30 min. The supernatant was discarded, and the cell samples were kept under the shade with a fan for 2 days (48 h) to evaporate the water remnant. The dried samples were grounded into a fine powder using a pestle and grinder with the help of liquid nitrogen. The grounded algae were dried for 30 min in an incubator at 80°C for releasing leftover water. Then the algae powders were stored in different jars for extraction experiments in a sealed container [17].

Extraction of oil for Biodiesel production

The lipid was extracted using the Folch extraction method [18-19] after the algae sample was dried (100%) and powdered. The procedure called for dissolving 1.50 mg of biomass in 10 mL of chloroform/methanol (2:1 v/v) and agitating the mixture vigorously for 30 seconds. Thereafter, the mixture was agitated at room temperature for 15-20 min. Cell debris was separated from the supernatant by centrifuging the mixture at 8000 rpm for 10 min. To clean this supernatant, we used a 0.9% NaCl solution and a vortex for a few seconds. For 5 min, the mixture was centrifuged at 3000 rpm.

The lipid-rich chloroform layer at the bottom of the tube was carefully scraped off and collected in a pre-weighted glass vial holding 20 milliliters. Using the same procedure as before, 5 mL of chloroform/methanol (1:1 v/v) were used three times to extract the residue. This is the same vial that contained the supernatant. After that, the solvent was dried in an oven at 65 degrees Celsius until the lipid's weight remained constant. An electronic weight balance was used to measure the amount of oil that was taken out. The oil yield (wt. %) was calculated as described by Chen *et al.* [20] and Arun *et al.* [21] by using the equation;

$$\text{Extracted oil efficiency (wt. \%)} = \frac{\text{Mass of oil extracted (grams)}}{\text{The total mass of dried algae}} \times 100$$

RESULTS AND DISCUSSION

Optimization of growth media and pH

It was observed that on day one the biomass concentration was statistically similar across all the media and pH levels except in MBG-11 at pH of 7 where the concentration was slightly higher. On day two it shows some variations where the MBG-11 at pH 8 gave the highest concentration but statistically similar with MBG-11 at pH 7 and M8 at pH 8 and a minimum concentration was recorded in M8 and N8 both at pH 6 as shown in **Table 1**.

Determination of Biomass Concentration

The highest biomass concentration of *C. vulgaris* was recorded in MBG-11 at pH 8 (0.68 mg/L/D) on day three, followed by BG-11 at pH 8, but they are statistically the same with MBG-11 at pH 7 and BBM at pH 8 (0.77 mg/L/D, 0.77 mg/L/D and 0.72 mg/L/D respectively). The least biomass concentration was recorded in N8 at pH 6 which is statistically similar with M8 at pH 6 and N8 at pH 7, (0.49 mg/L/D, 0.51 mg/L/D and 0.51 mg/L/D respectively). Averagely, at all the days, the highest biomass concentration was recorded in MBG-11 at pH 8 followed by BG-11 also at pH 8 and the minimum concentration was recorded in N8 at pH 6 (**Fig. 1**).

This can be explained by the fact that higher nitrogen concentration is favourable for increasing biomass growth as seen in MBG-11 media. However, M8 (having maximum nitrogen concentration) showed poor biomass concentration due to the deleterious effect of nitrogen at higher concentrations. Also, slightly basic condition is favorable for an increase in biomass concentration in *C. vulgaris* [22].

Table 1. Effect of pH and media on biomass of *C. vulgaris* (mg/L/D).

	Day One			Day Two			Day Three		
Media/pH	6	7	8	6	7	8	6	7	8
BBM	0.46^{b}	0.47^{b}	0.46^{b}	0.55^{bcde}	0.56^{bcd}	0.57^{bc}	0.56^{ef}	0.62^{cd}	0.72^{b}
BG11	0.45^{b}	0.44^{b}	0.48^{b}	0.49^{defg}	0.52^{cd}	0.53^{cdef}	0.59^{de}	0.64^{cd}	0.77^{b}
MBG11	0.45^{b}	0.66^{a}	0.50^{b}	0.47^{fg}	0.57^{bc}	0.61^{ab}	0.64^{cd}	0.77^{b}	0.86^{a}
M8	0.42^{b}	0.44^{b}	0.44^{b}	0.45^{g}	0.49^{defg}	0.56^{bcd}	0.51^{fg}	0.56^{e}	0.65^{c}
N8	0.40^{b}	0.44^{b}	0.47^{b}	0.45^{g}	0.48^{efg}	0.47^{fg}	0.49^{g}	0.51^{fg}	0.63^{cd}
LSD (5%)			0.12	0.07			0.05		

Key: LSD = Least Significance Difference.

	Day four			Day five			Day Six		
Media/pH	6	7	8	6	7	8	6	7	8
BBM	0.62^{fghi}	0.74^{defgh}	0.79^{de}	0.67^{def}	0.87^{cde}	0.92^{cd}	0.76^{hi}	0.98^{def}	1.08^{de}
BG11	0.65^{efghi}	0.78^{def}	1.00^{bc}	0.75^{def}	0.59^{f}	1.13^{bc}	0.79^{ghi}	1.00^{def}	1.26^{bc}
MBG11	0.68^{efghi}	0.86^{cd}	1.14^{ab}	0.78^{def}	1.00^{c}	1.35^{ab}	0.93^{efg}	1.12^{cd}	1.42^{ab}
M8	0.59^{hi}	0.64^{efghi}	0.77^{defg}	0.64^{ef}	0.75^{def}	0.88^{cde}	0.69^{i}	0.88^{fgh}	0.92^{efgh}
N8	0.57^{i}	0.61^{ghi}	0.74^{defgh}	0.61^{ef}	0.69^{def}	0.85^{def}	0.66^{i}	0.78^{ghi}	0.89^{fgh}
LSD (5%)			0.16	0.27			0.16		

Key: LSD = Least Significance Difference.

	Day Seven			Day Eight			Day Nine		
Media/pH	6	7	8	6	7	8	6	7	8
BBM	0.84^{ef}	0.98^{def}	1.14^{cd}	0.99^{efg}	1.01^{efg}	1.25^{ef}	1.04^{f}	1.19^{def}	1.48^{de}
BG11	0.96^{def}	1.02^{def}	1.47^{b}	1.03^{efg}	1.25^{ef}	1.78^{bc}	1.13^{def}	1.51^{cd}	2.02^{b}
MBG11	1.10^{cde}	1.37^{bc}	1.83^{a}	1.30^{de}	1.63^{cd}	2.11^{ab}	1.49^{d}	1.90^{bc}	2.47^{a}
M8	0.76^{f}	0.91^{def}	1.02^{def}	0.85^{g}	0.93^{fg}	1.12^{efg}	0.99^{f}	1.08^{ef}	1.25^{def}
N8	0.73^{f}	0.87^{def}	0.89^{def}	0.88^{g}	0.91^{fg}	1.04^{efg}	0.88^{f}	1.00^{f}	1.12^{def}
LSD (5%)			0.29		0.36			0.40	

Key: LSD = Least Significance Difference.

	Day Ten		Day Eleven				Day Twelve		
Media/pH	6	7	8	6	7	8	6	7	8
BBM	1.22^{efg}	1.49^{def}	1.90^{bcd}	1.27^{efg}	1.52^{de}	1.97^{bc}	1.26^{gh}	1.51^{fg}	2.05^{cd}
BG11	1.38^{efg}	1.78^{c}	2.24^{b}	1.49^{def}	1.79^{cd}	2.24^{b}	1.50^{fg}	1.88^{de}	2.46^{b}
MBG11	1.62^{de}	2.07^{bc}	2.68^{a}	1.74^{cd}	2.12^{bc}	2.82^{a}	1.95^{de}	2.34^{bc}	3.01^{a}
M8	1.12^{fg}	1.34^{efg}	1.48^{def}	1.14^{fg}	1.39^{efg}	1.57^{de}	1.20^{gh}	1.40^{fgh}	1.62^{ef}
N8	0.98^{g}	1.00^{g}	1.13^{fg}	1.02^{g}	1.12^{fg}	1.28^{efg}	1.05^{h}	1.16^{gh}	1.45^{fg}
LSD (5%)	0.43		0.37				0.35		

Key: LSD = Least Significance Difference.

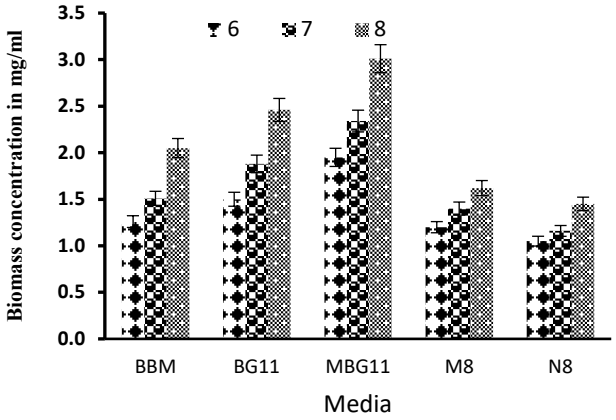

Fig. 1. Biomass concentration in different media and pH.

Extraction of oil for Biodiesel production

The Lipid content was reported as a percentage of lipids to biomass DW. Similar to biomass concentration, significant differences in lipid content were found across all the pH and culture media (**Fig. 2**).

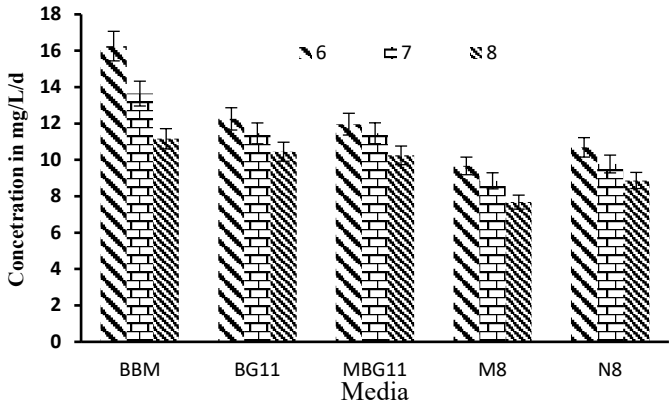

Fig. 2. Oil concentration in different media and pH levels.

The highest lipid content of 31.22% from *C. vulgaris* was recorded in BBM at pH 6, then at pH 7, followed by BG-11 at pH 6 and MBG-11 at pH 6 which are all statistically similar (26.2, 23.53 and 22.97%, respectively). The least lipid content was recorded in M8 at pH of 8 and pH of 7 with corresponding lipid content of 14.75% and 16.99%, respectively, and N8 at pH 8 with corresponding lipid content of 17.02% which all are statistically similar as shown in **Table 2**.

Table 2. Percentage (%) Effect of pH and media on oil production of *C. vulgaris.*

Media/pH	6	7	8
BBM	31.22^{a}	26.20^{b}	21.43^{cdef}
BG11	23.53^{bc}	22.01^{cde}	20.07^{defg}
MBG11	22.97^{bcd}	22.01^{cde}	19.68^{efg}
M8	18.57^{fg}	16.99^{gh}	14.75^{h}
N8	20.52^{cdef}	18.77^{efg}	17.02^{gh}
LSD (5%) 3.26			

Key. LSD = Least Significant Difference.

Furthermore, the accumulation of lipids in the algal biomass under nitrogen deficiency conditions corroborates these findings [22]. Several factors, including the desired end product, growth rate, and medium cost, should be considered before settling on a specific culture medium. Nitrogen, however, is the most important component of the growth medium and a limiting nutrient for microalgal biomass growth and lipid productivity. When comparing lipid content at different pH values and different culture media, BBM and BG-11 at pH 6 produced the highest value, while M8 medium and N8 produced the lowest. Unfavorable culture conditions, such as nutrient starvation, tend to increase the lipid content of microalgae [9, 23]. Microalgae species' lipid yields can be increased by at least 300% under nitrogen-limited conditions, as reported by Li *et al.* [24]. As previously stated, BBM and BG-11 are the recommended growth media for microalgae in this investigation due to their ability to maximize lipid production. In contrast to the BG-11 medium, which has good potential to increase lipid productivity by applying nitrogen-deficient conditions, the BBM had higher lipid content because of relatively lower nitrogen and phosphate concentrations.

In addition, it was calculated that the total cost of chemicals to prepare MBG-11 medium was Rupees (Rs) 0.784, while the total cost of chemicals to prepare BG-11 and BBM growth media was Rs. 0.472 and Rs. 0.094, respectively (Rufees outlay is a formula for calculating index number). The expense of preparing MBG-11 can be cut by as much as Rs. 0.460 if it is used to cultivate algae in a nitrogen-deficient medium (assume half concentration of nitrogen in the case of MBG-11). Total lipid productivity will decrease if the nitrogen concentration in BBM, which is already low, is further reduced. Consequently, MBG-11 had been considered as the best medium in this study and can be used as a culture medium for further research due to its potential for increasing lipid productivity and decreasing costs.

CONCLUSION

The results revealed that *Chlorella vulgaris* showed good potential for lipid production in all the medium and pH level. However, maximum biomass concentration of the *C. vulgaris* was observed in MBG-11 at pH 8 amongst all the tested medium and pH levels. On the basis of lipid production, it was observed that BBM growth media at pH 6 yielded the highest percentage of oil produced. Alternative fuel options, such as biodiesel made from algae biomass, are on the table due to the fuel's accessibility and environmental friendliness. Microalgae have the potential to generate a sizable quantity of biodiesel (*C. vulgaris*). By extracting its oil, *Chlorella vulgaris* can then be converted to biodiesel via a trans-esterification reaction, making it a viable feedstock for the biodiesel industry.

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