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Evaluating the impact of different growth media on Chlorella species for biomass production and biochemical composition

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ABSTRACT

The quest for ecologically and economically viable algal production necessitates research into the biological responses of algae under diverse growing environments. This study uses a semi-continuous culture with three growth conditions (BG-11, Chu 10 and Bold basal media) to look at metabolic components and biomass generation of Chlorella species. The highest cell density obtained in BG-11, Chu 10 medium and BG11 had the highest yields of the pigment "Chlorophyll a" as well as theprotein and carbohydrates. The Chu medium analysis shown the highest yields and concentrations of various lipids including phosphate lipid (PL),acetone mobile polar lipids (AMPL), steroid ester, and hydrocarbon compounds. In the BG11medium, the levels of omega-3 oils, sterol (ST), and triglycerides (TAG) were maximum. Since BG-11 provided the highest growth efficiency and biomass output without the requirement for vitamins, which was associated with other growth media, it is the most economical choice for vegetating.

Key words:CHLORELLA SP, CHLOROPHYLL, MICROALGAE, GROWTH MEDIA, BIOMASS.

INTRODUCTION

For the commercial development of biofuels and bioproducts from algae to be profitable and sustainable, it is imperative that significant advances in biomass productivity and the related biochemical composition be addressed. By utilizing methods that combine engineering principles with the basic biology of photosynthetic organisms, we can gain a deeper comprehension of the simple relationship between the growth rates, productivity, and composition of algae.A prospective feedstock for the long-term, sustainable synthesis of several high-value chemicals and products such as fuel, feed, and food is algae biomass. Effective valorization techniques including bio-refining mechanism are crucial for increasing the retail price of microalgae metabolites. With their abundance of anti-inflammatory, anti-microbial, anti-oxidant, and tumor-fighting, and other medicinal and regenerative qualities, algae-based extracts provide outstanding resources for biotechnology viablecompounds [1].Comprehensive data pertaining to the generation of fucoxanthin and biomass, including molecular and metabolic investigations, is currently lackinggenetic and metabolic processes. Research should be the focus of future studiesmechanisms using fucoxanthin under these circumstances, expanding the production of fucoxanthin , the financial and environmental evaluations. Synthetic production of fucaxanthinin order to develop sustainably industrial fucoxanthin extraction [2].The synthesis of fucoxanthin from algae has been summarized in a number of haphazard narrative reports [2]. A comprehensive evaluation could effectively reduce bias and improve the validity of each study's conclusions. Still, there aren't many systematic evaluations that concentrate on fucoxanthin [3]. The majority of marine algae use theBenson-Calvin cycle to ingest CO_2, Nevertheless, multiple species exist also use the Cycle of hatch & slack to improve the photosynthesis procedure efficiency [4]. The supernatant of pig slurry cultures had greater quantities of extracellular polysaccharides (EPS), which showed a pattern of lower viscosity trend as in contrary to biomass content percentages, significantly greater quantities found in pig slurry cultures. The fluidity of biomass from microalgae species is significantly influenced by the culture medium and extracellular polymeric substances (EPS). This highlights the necessity for further research to facilitate and optimizesophisticated intermediary procedures in context of a sustainable economy that is circular as well as achievement of the 2030 Agenda for develop Sustainably[5].Because of its versatility in a number of industries, including cosmetics, pharmaceuticals, food and feed for humans and animals, biomass, capturing carbon dioxide(CO_2) along with wastewater sustenancerecuperation, micro algal biomass has distinguished itself as having tremendous promise as a sustainable alternative source [6]. Algal biomass has the potential to enter a number of biotechnological and agricultural sectors that produce goods that are useful to people and latestaccessible things. This position into the world efficiencymarketplace is expanding annually, demonstrating the enormous potential of green technologies [7]. The production of algal metabolites significantly aided in recent years by the development of modern technologies e.g. genetic science, integrated Phonemics, the industrial sector, and the treatment of biomass from algae [8]. Many strategies for reducing these costs have been suggested.There are several suggestions for reducing these costs, some of which include using wastewater and factory waste. Consider this behavior as a biotech characteristic that may be manipulated to control the growth and chemical framework of the algae, with an emphasis on greater efficiency and certain chemical compounds. That's why it's interesting to select affordable and effective media to increase the quantity of algae production. Harrison and Berges (2005)

proposed that three basic types of experiments can be used to determine the best production conditions: Algal biomass yield, culture maintenance, and physiological/growth assays. The growth rates for the microalgae in culture medium give a wide indicator of their physiological status and health, since they demonstrate how the algae metabolizes the nutrients it receives in response to each cell cycle[9]. This revelation clearly shows that batch cultures are only able to offer microalgae favorable growth conditions for a brief amount of time before the cell's metabolic pathways break down and photosynthesis decreases (Lombardi and Several culture systems have been suggested as batch culture substitutes for the cultivation of algae biomass and physiology research [10]. Because this method provides a reliable source of microalgal biomass, the capacity to control the biochemical properties of both continuous and semi-continuous cultures has raised concerns [10].This work examined the metabolic makeup of the microalgae Chlorella species by means of "semi-continuous "media that offer favorable circumstances for development at an equilibrium level for peptides, lipids, and sugars. Using Iatroscan TLC-FID, lipid class analysis was performed on the quantity of biomass conserved in different growing mediums. The results showed that Chlorella species tested under healthy growth conditions exhibited different molecular constituents (fats, amino acids, as well as starches) along with development percentages across the course media means related to culture source studied.

Material and method

The Chlorella species strain (the freshwater microalgae culture) was collected from Centre for Conservation and Utilization of Blue green algae, ICAR–Indian Agricultural Research Institute New Delhi, India.It was grown in three different conditions in the laboratory: partially concentrated Chu 10 (Chu) [11], Bold Basal culture[12]and BG-11 medium [13].

Analytical Method

A regulated dark/light cycle (8:16 hours) with luminosity of 150 µmol/m^2/s was applied to 250 milliliters sample contained in 500-milliliter polycarbonate flasks at 20°C.Chlorella species cells were prepared for the studies, by acclimating them to the particular growth medium in batch culture method. In this study acclimation comprisedculture transfer at exponential growth, linked with an increasing rate of growth of each new culture was observed. In the culture media to be examined the microalgae had been considered acclimatize and displaying each treatment concentration after three successive growth rates that are statistically comparable. The semi-continuous experimental cultures started from this state andall treatments started with a cell count of about 10^5cells/ml. Removing or replacing growing media that has an equivalent composition of new medium to maintain the rate of cell density throughout the experiment to maintain semi – continuous culture.Spectrophotometric measurements were made of all the culture's growth using an "Analitica Jena" spectrophotometer at 684 nm. Based on the equation belowthe specified rate of growth (µ) is determined for semi-continuous culture system.

$$\mu = \log (ABS (t_2) / a \times ABS (t_1) (t_2 - t_1)^{-1} \quad (1)$$

$$a = \text{total volume} - \text{Removed volume} / \text{Total volume} \quad (2)$$

Here ABS = Absorbance, t = Time

The cell counts were made under an optical microscope for determination of cell densities. Dried weight of the algae was calculated by gravity measurement using a Sartorius MC21S analytical scale (Precision Weighing Balances, Bradford, MA)and bimolecular yield (Q_V) was calculated as follows-

With an upgraded bright-line Neubauer hemocytometer, cell counts were made under an optical microscope for calculating cell densities. Making use of filters made of $C_{76}H_{114}O_{49}$ (cellulose acetate)(Millipore, Brazil) has an aperture diameter of 0.45 micrometer with drying time of 24 hours at 60°C , dried weight of the algae was calculated by gravity measurement using a ABC INC analytical scale (Precision Weighing Balances). By multiplying the concentration that was present per cells throughout the sample period taking into account one specific growth rate, bimolecular yielding (QV) was calculated.

$$Q_V = \mu X \quad (3)$$

Let X be the bimolecule percentage in pg/cell where "μ" indicate average daily growth rate. The experiments were done in three replicates. The media was sterilized for 30minutes at 121°C by Digital autoclaving it afterwards its pH value was adjusted to 7.

Analysis of chlorophyll a

Centrifugation was done at 12,000 rpm for 15 minutes to create algal pellets. A solution of 70% acetone (6 ml) was added to the pellet, and kept at 4°C for 12 hours. After centrifuging the sample once again, the absorbance of the supernatant was measured at 645 and 663 nm. The following principle was used to find the total chlorophyll concentration [14].

Chlorophyll (μg/l) = 8.02 X OD663 + 20.21 X OD645.

Protein and carbohydrates Estimation

The method proposed by Lowry et al [15] was used to calculate the total amount of "protein" of the strains. The following reagents were prepared:
Reagent **I**1N solution ofNaOH

Reagent **II**(i) Na_2CO_3 solution solution 2%

(ii) $CuSO_4.5H_2O$ solution 0.5% (w/v) in 1% (w/v) of Na–Ktartarate.

2ml (ii) was mixed with 48ml of (i) prepared immediately before use.

Reagent **III** Folin-Ciocalteau reagent: Diluted the reagent with water in equal amount

Reagent **IV** Standard protein: Bovine serum albumin solution was prepared in the range of 10-100 μg ml^{-1}

Procedure- An equivalent volume of 1N NaOH was added to the extracted sample (0.5 ml), and heat for for five minutes. Reagent II (2.5ml) is added to this solution and left for 10 minutes at room temperature. After that, 0.5 ml of reagent III was quickly added and well blended. After an interval of fifteen minutes, the blue solution's absorbency was measured at 650 nm in a

spectrophotometer. Intracellular carbohydrates were calculated using the modified phenol-sulphuric acid method [16], with glucose serving as a standard.

Assessing the quantity of fats

The extraction of lipids was done by a modified method reported by Parrish 1999 [17]. In this method a mixture of CH_2Cl_2, CH_3OH, and $H_2O$was used in 2:1:0.75 ratios. GF/C filtration systems, which had been already kept at 400° C within a muffle oven, were used to filter samples (50 ml). The filtered samples were added to a known amount of Hexadecan3-one. After that, the filtrate was centrifuged and sonicated for two minutes. The calibration curve have been prepared for following nine lipid standards to identify the peak. Three replicates of each analysis were performed. The Iatroscanwas used for lipid class analysis at173 ml/min for hydrogen stream, 2 L/minute for flow of air, and 4 mm/s for scan speed.

Table **1 - List of lipids used as standard**

List of Lipids	
1-hexadecanol	free aliphatic alcohol
Cholesteryl palmitate	Wax esters/steryl ester
n-Hexdecan-3-one	Ketone
Palmitic acid	free fatty acids
n-Nonadecane	Aliphatic hydrocarbon
Cholesterol	Free sterol
Gyceryltripalmitate	Triglyceride
1-monopalmitoyl-rac-glycerol	Acetone mobile phase lipids
1,2,di-0-hexadecyl-snglycerol-3-phosphatidylcholine	Phospholipids

Table **2- Biochemical constitution of "Chu-10 medium [11].**

Component	gm/l	Quantity use	
Calcium nitrate $Ca(NO_3)_2.4H_20$	57.56	1 ml	
Dipotassium Phosphate (K_2HPO_4)	10.00	1 ml	
Magnesium Sulphate ($MgSO_4 \cdot 7H_2O$)	25.00	1 ml	

Sodium Silicate (Na_2SiO_3)	5.0	10 ml	
Sodium Carbonate (Na2CO3)	20.00	1 ml	

Table 3- Trace Elements solution for Chu 10 medium [11]

Name of Component	gm / 100 ml use	Quantity Use
Ferric ammonium citrate $(NH4)_3$ [Fe(C6H4O7)]	6.00	1ml
Citric acid	6.00	1ml
Boric Acid (H_3BO_3)	2.86	1ml
CopperSulphate($CuSO_4.5H_2O$)	0.079	1ml
Cobalt Nitrate $Co(NO_3)_2.6H_2O$	0.0494	1ml
($NaMoO_4·2H_2O$)Sodium molybdate	0.390	1ml

Table – 4 Chemical Composition of Bold Basal Medium [12]

Name of Chemical Component	'Stock solution' (gm in 100 ml Water)	Quantity use
Sodium nitrate ($NaNO_3$)	2.5 gm	10 ml
CalciumChloride ($CaCl_2·2H_2O$)	0.25 gm	10 ml
MagnesiumSulphate ($MgSO_4·7H_2O$)	0.75 gm	10 ml
DipotassiumPhosphate (K_2HPO_4)	1.75 gm	10 ml
PotassiumPhosphate (K_2HPO_4)	1.65 gm	10 ml
Sodium Chloride (NaCl)	0.25 gm	10 ml
Alkaline EDTA solution		1 ml
EDTA	5.00 gm	
Potassium Hydroxide(KOH)	3.1 gm	
Acidified Fe solution	-	1 ml
Boric acid (B_2O_3)solution	1.142 gm	1 ml
Trace Element solution	-	1 ml

Acidified Fe solution: 0.1 ml H_2SO_4 dissolve in 100 ml dH_2O, followed by 0.498 gm $FeSO_4.7H_2O$ added.

Table 5- Trace Elements solution for Bold Basal medium:

Name of Component	gm / 100 ml use	Quantity Use
ZincSulphate ($ZnSO_4 \cdot 7H_2O$)	0.882	1ml
Managenous chloride($MnCl_2 \cdot 4H_2O$)	0.144	1ml
Molybdenum Oxide(MoO_3)	0.071	1ml
CopperSulphate($CuSO_4.5H_2O$)	0.157	1ml
Cobalt nitrate hexa - hydrate($Co(NO_3)_2 \cdot 6H_2O$	0.0494	1ml

Table 6 - Chemical composition of BG_{11} Medium [13]

ComponentStock	Stock Solution (gm/100ml)	Quantity Use
Sodium nitrate ($NaNO_3$)	75 g /500 ml	10 ml
Dipotassium Phosphate (K_2HPO_4	4.0 g / 100 ml	1.0ml
Magnesium Sulphate ($MgSO_4 \cdot 7H_2O$)	7.5 g /100 ml	1.0 ml
Calcium Chloride ($CaCl_2 \cdot 2H_2O$)	3.6 g /100 ml	1.0 ml
Citric acid ($C_6H_8O_7$)	0.60 g / 100 ml	1.0 ml
Ferricammoniumcitrate$(NH4)_3$ [Fe(C6H4O7)]	0.60 g / 100 ml	1.0 ml
EDTA (disodium salt)	0.10 g / 100 ml	1.0 ml
Sodium Carbonate (Na_2CO_3)	2.0 g / 100 ml	1.0 ml
Trace metal (mix A5)	1.02 ml / 100 ml	1.0 ml
Agar	15.0 g /l	1.0 ml
Mineralized water	2.0 L	

pH supposed to be 7.1 subsequent to sterilization

Table – 7 Trace Element mix A5

Component	Quantity Used (gm /l)	Quantity Use
Boric Acid (H_3BO_3)	2.86 g	1 ml
Managenous chloride ($MnCl_2 \cdot 4H_2O$)	1.81 g	1 ml
Zinc Sulphate ($ZnSO_4 \cdot 7H_2O$)	0.22 g	1 ml
Sodium molybdate ($NaMoO_4 \cdot 2H_2O$)	0.39 g	1 ml
Copper Sulphate ($CuSO_4 \cdot 5H_2O$)	0.08 g	1 ml

Cobaltnitrate hexahydrate [$Co(NO_3)_2 \cdot 6H_2O$]	0.05 g	1 ml
Distilled water	1.5 L	1 ml

8FREE ION CONCENTRATIONS

It is anticipated that there would be variations in the availability of nutrients, particularly trace metals, since "culture media composition' differs. We determined the free ions concentration of zinc (Zn), copper (Cu) in addition to iron (Fe), which serve as vital trace elements for algal metabolism. We used chemical equilibrium software MINEQL$^+$ for this determination.

Analysis of Data

To determine the growth parameter, biochemical composition and biomass production, ANOVA with Tukey's HSD post hoc test were applied. The statistics software "PAST version 2.09" for Windows had been employed to do the analysis of principal components, whereas SPSS version 17 for Windows was deployed for carrying out an ANOVA.

RESULTS

According to equilibrium chemical calculations the most minimal 'liable and free' levels of Cu found in the BBM medium as expected due to inclusion of EDTA. BG11 had the highest values of the speciation of Cu, Zn, and Fe while BBM had the lowest values. On the other hand, Chu 10 medium showed intermediate concentrations for both labile as well as free metal ions. Table 8 shows the MINEQL$^+$ findings of metal speciation for Fe, Cu, and Znin growth-promoting media. The values are in pCu, pFe, and pZn, where p is the negative base of $\log_{10}$of molar concentrations.

Table – 8

Name of metal	Speciation	BG-11	Chu10	BBM
Copper(Cu)	FREE	7.76	9.56	12.84
	LIABLE	8.03	9.51	8.72
IRON(Fe)	FREE	5.52	5.62	5.89
	LIABLE	6.05	7.09	6.96
ZINC(Zn)	FREE	7.03	9.11	10.03
	LIABLE	8.53	10.80	11.49

Fig. 1 displays the results for biomass.

Fig. 1 (a)

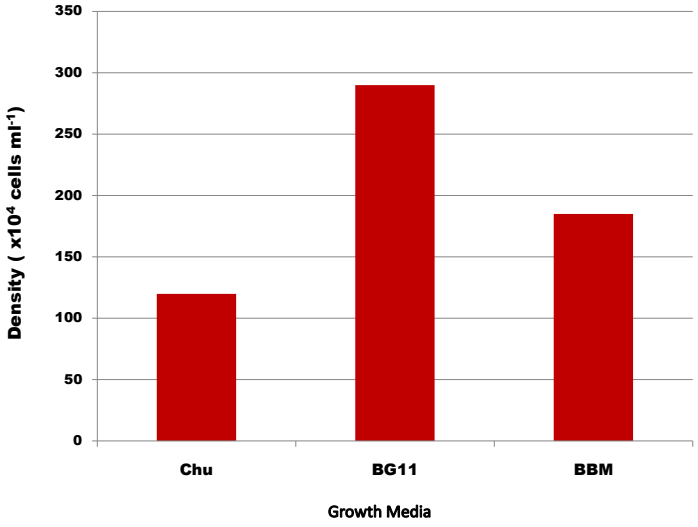

Fig. 1 (b)

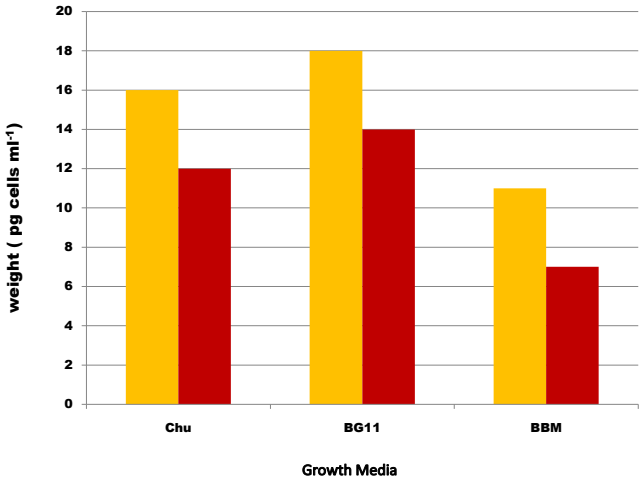

Fig. 1 (c)

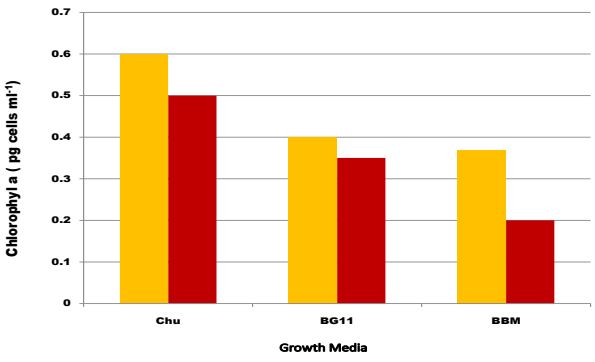

Fig (1) - (a) cell density (cells/ml); (b) dry weight (pg /cell), and (c) chlorophyll a production (yellow bars) and yield (red bars) (pg /cell) for Chlorella species full-grown in Chu, BBM, BG_{11} medium. Error bar signify typical divergence designed for n = 3

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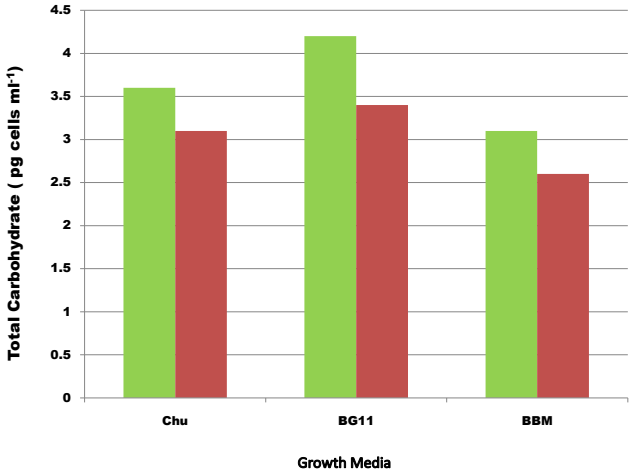


Fig. 2 (a) Total carbohydrates (green-production and red- yield)

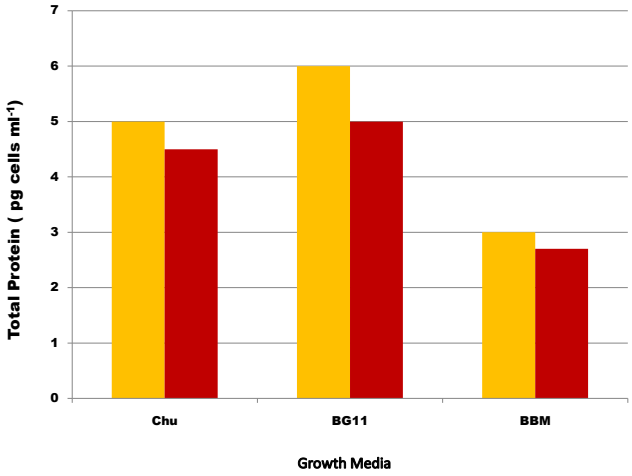


Fig. 2 (b) Total protein (yellow-production and red- yield)

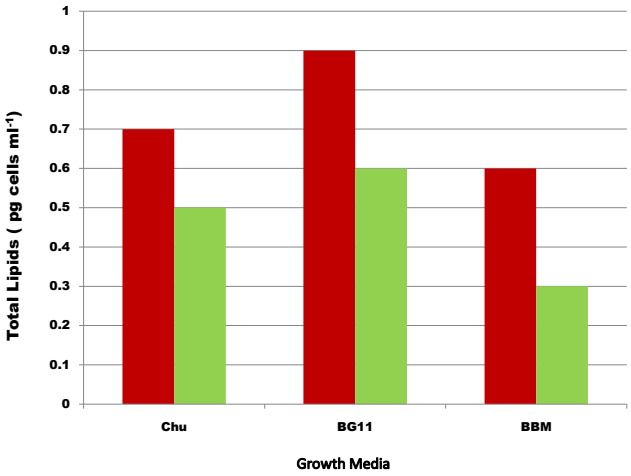


Fig. 2 (c) Total lipids (red-production and green-yield)

Fig. 2. displays the results for total carbohydrates, proteins and lipids. Total carbohydrate (a), total protein (b) and total lipid (c) production (black bars) and yield (gray bars) (pg.cell-1) of Chlorella Sp.as a function of media type using semi continuous culture system. Error bars represent standard deviation for n = 3. BG11 = BG11 medium,, Chu = Chu medium, BBM = Bold Basal medium

The Chu medium had the highest concentrations and yields of the hydrocarbon group, steryl ester, fatty acids, aliphatic alcohol, acetone mobile phase liquid and phospholipids, while the BBM medium had the lowest value. The BG11 media and Chu 10 medium did not exhibit a significant difference ($P < 0.05$) in terms of lipid class composition or total lipid production.68% of the variation seen in the PCA could be explained by the first two components, according to principle component analysis. The amounts of both phosphorus and nitrogen had a positive correlation with the quantities of TAG, 'ALC', 'FFA', 'SE', & 'ST'. Furthermore, P showed a significant ($p <0.05$) correlation with Figure 3, Lipid type Percent component of Chlorella sp. cultivated in various growth media. Polar lipids are denoted by AMPL and PL, while lipids that are neutral are denoted via HC, SE/WE, TAG, FFA, ALC, and ST." Standard deviation is shown by error bars for n = 3. Chu is equal to Chu medium, BBM to BBM medium, and BG11 to BG11 medium. Classes PL and AMPL are on the right axis. Concentration of carbohydrates, total lipids, AMPL, chlorophyll a, dries weight, and cell density. A positive correlation was seen between AMPL levels and chlorophyll a concentrations. Relationships between the concentrations of each structure lipid group (PL) and the type of storage lipid category (TAG) were inverse.

DISCUSSION

AGROPECTION AND BIOMASS Biosynthesis

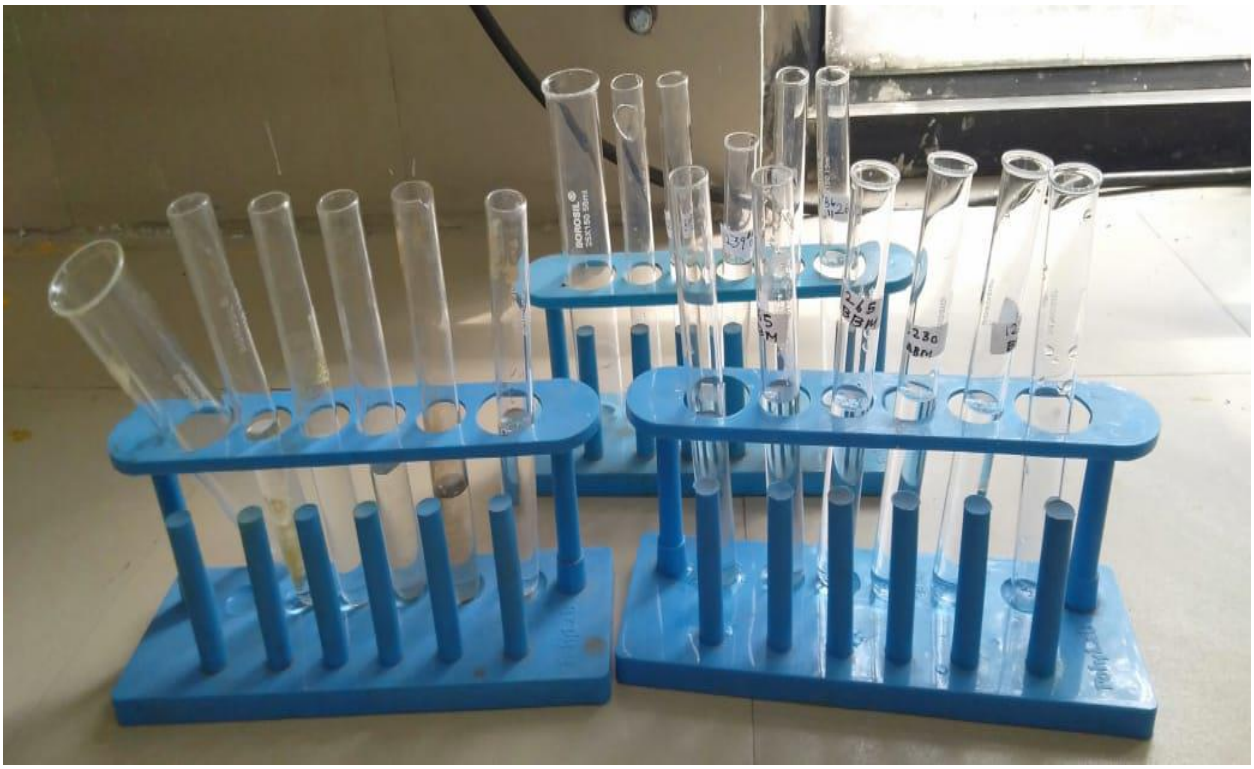
The rate at which Chlorella species advancing in BG_{11} medium be found to be substantially higher (0.84/d) than in the other two culture media examined, indicating that the type of media used had an impact on growth performance. This figure of the expansion ratefor Chlorella sp. contained by the range of values described in the literatureRegarding Chlorella sp. cultivated in f/2 media [18]obtained specific growth rates of 0.40-0.85/d, which are greater than that reported in [19].for

C.species .semi-continuously (0.40/d) in supplemented C medium.BG_{11} had nearly twofold(2.74 x 106 cells / ml) the cell density ofthe extra growth mediums that we examined, indicating that cell density, an important biomass characteristic, shifted substantiallyamongst mediums. All of the studies that looked at the growth of C. species in controlled environments, including [20].are comparable to our findings, though. According to our research, the highest amount of chloroplast produced by C. species in the cultures was 1 µg / ml,It is known that the growth conditions of phytoplankton cells affect their ability to produce chlorophyll a Chinnasamy et al. (2009) [20].identified that specific group ,various cultural groups of C. species multipliedon"BG 11 media" generated maximal chlorophyll content of around 5 µg / ml during their initial ten days period. These values were much below whatever was documented here. In contrast to Bertoldi et al. [21].the current data demonstrate a significant yield with the Chu medium culture (5.9 x 10-13 g/cell), if the results are expressed as chlorophyll concentrations cell unit rather than per volume. The largest amount of chlorophyll a that these authors were able to produce in C. species cultivated in bold basal media (BBM) media was 2.8 x 10-13 g/cell. "BG_{11} medium's" composition may have contributed to its maximum dried weight (16.82 pg/cell) concentrations attained because it included the highest amounts of N and P. Concentrations of proteins and carbohydrates were correlated with media elements such as phosphate, nitrate, and free copper ions. While greater than earlier research, the amount of protein synthesized by 50% (7.0 mg/l) in "Chu 10 media" and 6.8 mg/l in bold basal medium (BBM) is comparable to Bertoldi et al. [21].findings. C. species grown in BBM was found to have a percentageof amino acidsof roughly 52.3% (dry matter) by Bertoldi et al.[21].But it was demonstrated by Mahasneh [22].that "Chlorella strain" grown in a condition comprising 0.25 mg.15.7% protein was found in L-1 phosphate, whereas 37% protein (dry weight) was found in phosphate concentrations as high as 0.50 mg/l in comparison to Illman et al.[23], that found that C. species produced 29% (dry weight) of the protein in Watanabe medium, the findings we obtained of the amount of protein in Chu and BBM media are around twice as high. Under the current circumstances, C. species produced the most Saccharides (7.36 µg /ml) in the BG_{11} culturescomparable to the findings released by Habib et al. [24].are our results. We measured the equivalent of 33.74 g per 100 g of C. vulgaris cultivated in Chu media, compared to 30.8 g per 100 g of C. species produced in nitrogen, phosphorus, potassium (N.P.K)fertilizer supplemented growing medium as reported by these authors compared to the highest value of 37% we found for the same algae in BBM media.Illman et al.[23].recorded saccharidescontent for C. species in Watanabe medium of up to 50%. Our research results indicate that when suitable culture conditions are employed, semi-continuously produced cells can be utilised.According to Ferreira et al.[24], elevatedyield maybe maintained for extended period of time, and the biomass of microalgae cells that is produced will have a consistent metabolic content which can be subsequently adjusted by adjusting culture and ambient factors to raise its nutritional value. Not every lipid class was impacted through the growth medium employed in terms of lipid production. Less than 5% of the total lipid synthesis was made up of sterol esters (SE), "FFA" (free fatty acids), free aliphatic alcohol (ALC), and aliphatic hydrocarbons (HC). This percentage falls within the typical range that most microalgae should fall within in a healthy growing environment, regardless of the culture media (Hu 2004, US DOE 2010, Scarsella et al. 2010).[25].High FFA readings (more than 5%), as suggested by Lombardi and Wangersky [26]. are most likely the product of lipases that were released when the sample was being prepared, and it required being interpreted cautiously. The

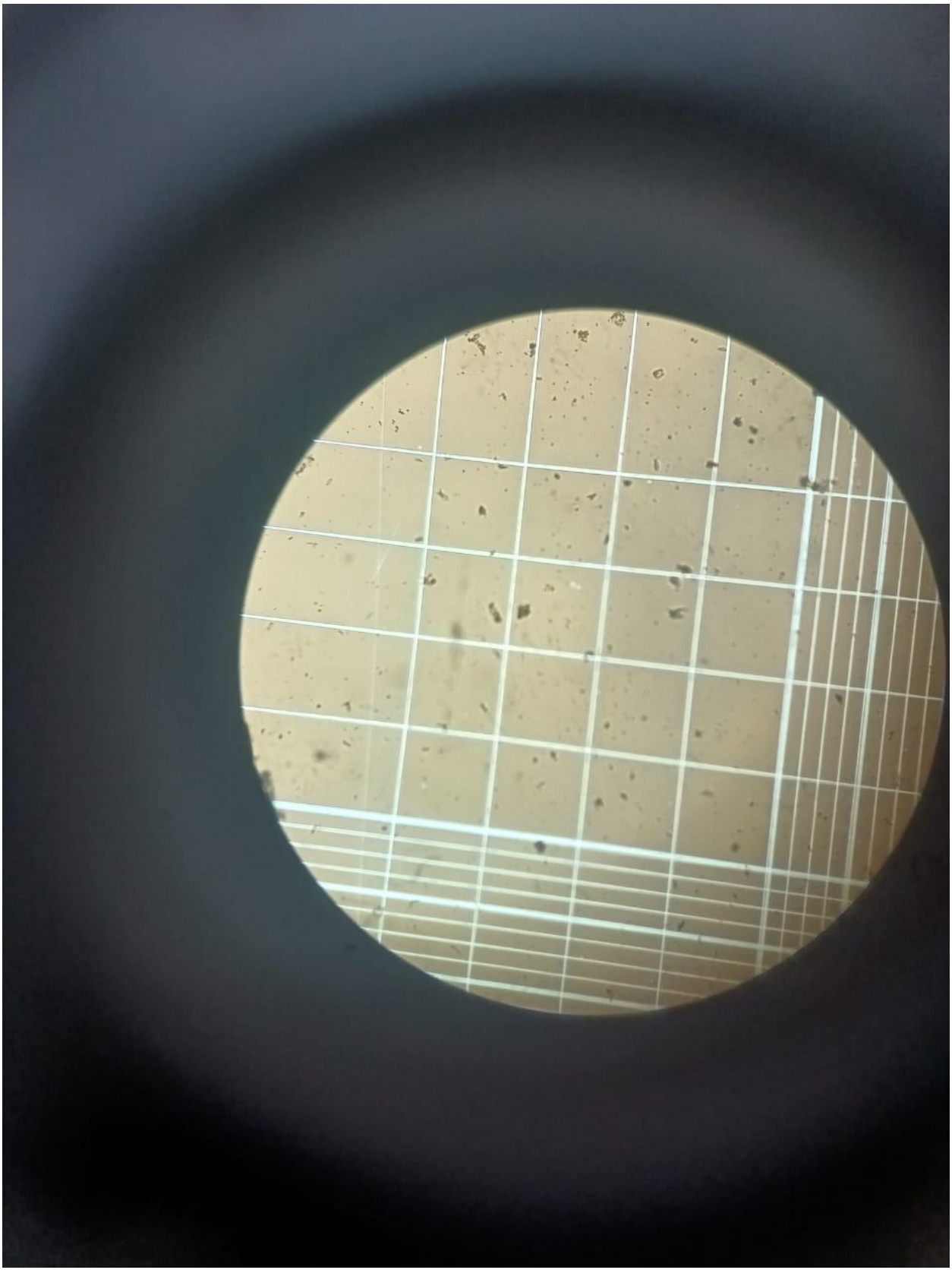
study's culture media evaluated varied in total lipid synthesis; the lowest total lipid production was seen in C. species grown in BBM, indicating that the cells were growing in a healthy environment. The N: P proportions in culture substrate, which were Chu 10:1, BG11 10:1, and BBM 100:1, for the ones utilised here, have been shown in the literature to have an impact on total lipid production. According to Griffith and Harrison [27] and Pittman et al. [28], provided compelling evidence of this phenomenon by demonstrating a growth inDunaliellatertiolecta's overall lipid synthesis within N: P ratios in the range about underneath 15:1, whereas this was not at 30:1. This finding validates the significance in media attributes on the phytoplankton's metabolic compositionand alters the calorie content of them. We discovered that the Chu medium had the highest percent total lipid yield, yielding roughly 10.7 g lipids per 100 g dry algae. This is roughly twofold the amount of 5.9 g the lipids each one 100 g dry out algal cells stated by Converti et al. (2009) [29]. for C. species emerged during batch culturesafter a period of fourteen days, applying “Bold's Basal Medium (BBM)”Yet, lipid yield of 1.5 mg / lt /d observed in the Chu and BG11 media was less than that of C. species cultivated in the newly modified Fritzgerald medium described by Widjaja et al[30]which was around 4.2 mg / lt / d.

CONCLUSION

BBM (Bold Basal Medium) medium produced the lowest overall output of Chlorella species for the most of the parameters examined when compared to other treatments; BG_{11} cultures with the highest cell density were found to have higher system productivity overall. This indicates that because BG_{11} doesn't contain vitamins, it can be a more affordable and efficient media option than the others. The biomass's biochemical makeup varied significantly between the various media types, and in certain instances, notable variations were found.Chu 10 and the BG_{11} medium produced the highest amounts of biomass production in terms of total carbs and lipids. The physiological, immunological, and growth functions of nourishment for herbivorous creatures “ chlorella species“ may benefit from the increased concentration of polar lipid classes (PL, phospholipids, and AMPL, acetone mobile polar lipids) found in Chu 10 medium.



Pictures of 1, 2, 3 showing different stages of different culture media.



Pictures 4,5 showing Neubauer Hemocytometer

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