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COMPARATIVE ANALYSIS OF BIOMASS AND MACROMOLECULES COMPOSITION AMONG MICROALGAE UNDER PHOTOAUTOTROPHIC CONDITIONS

Biological Science

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ABSTRACT

Back ground: Microalgae are efficient photosynthetic potential can synthesize and store a large amount of lipids and carbohydrates. High-value renewable energy source such as biodiesel, bioethanol, and biogas are being were generated using these microalgae. **Objective:** The macromolecule composition of *C. pyrenoidosa*, *C. sorokinana*, *D. asmatus*, *S. acutus*, *C. reinhardtii*, and *C. vulgaris* was investigated in the study. **Results:** The average protein, carbohydrate, and lipid macromolecular composition of nutrient-sufficient exponential stage microalgae are 45.21 to 52.29 %, 15.14 to 22.91 %, and 12.70 to 19.78 % of dry weight, respectively. Total chlorophyll and carotenoids contents are in the range of from 16.78 to 23.32 g/ml and 1.22 to 2.9 g/ml, respectively. The microalgae *C. sorokiniana* (1.02±0.021) produced the higher biomass, while *S. acutus* (0.79±0.05) produced the lowest content. The *C. vulgaris* and *C. pyrenoidosa* had the highest and lowest cell density and cell number, respectively. **Conclusion:** The primary biomass, protein, lipids, carbohydrates, and pigment content results can be used to determine the viability of large-scale biomass production for biomass energy.

KEYWORDS

Algae, Biomass, Macromolecules, Renewable energy biomass energy/bio-energy.

INTRODUCTION

Bio-energy is a promising alternative to fossil fuels as it has the capacity for the reduction of CO_2 by keeping clean environment[1]. Sugars, grains, and seeds are first-generation biofuels, while lignocelluloses are second-generation biofuels, and microalgae were considered to be third-generation biofuels. Microalgae have a higher growth rate potential than crop plants, and their oil yield per unit area is 7 to 31 times higher than the next best palm oil crop, and algal oil processed into biodiesel, is comparable to crop oil derivatives.

Microalgal biomass was found to have large quantities of unsaturated fatty acids, food particles, and other valuable materials. Lipid (10-20%) content of microalgae differs from species to species and used in biofuel and dietary supplements[2]. The composition of biomolecules and minerals varies between species of algal species and also depends on growth conditions such as temperature, light intensity, pH, salinity, and medium nutrients[3]. Different algal species feature varied high growth rate or biomass yield is typically determined by their ability to double their biomass in a short period, such as 24 hours, during the exponential growth process[4].

Several studies were conducted to better understand the growth rate and macromolecule composition of various micro-algae organisms under autotrophic conditions. The goal of this research was to compare the biomass and macromolecule composition among *Chlorella pyrenoidosa, Chlorella sorokiniana, Desmodesmus armatus, Scenedesmus acutus, Chlamydomonas reinhardtii, and Chlorella vulgaris* under photoautotrophic conditions. Algal growth and biochemical parameters such as growth rate, biomass, lipid, carbohydrate, and protein were assessed as part of this research/study.

MATERIALAND METHODS

Growth and culture conditions

C. pyrenoidosa, C. sorokiniana, D. armatus, S. acutus, and C. vulgaris microalgae strains were collected from the National Collection of Industrial Microorganisms (NCIM), CSIR-National Chemical Laboratory, Pune, India and they were maintained in minimal TAP (Tris–acetate phosphate) media[5] at 25°C with a photon flux density of 50 μmol photons m^{-2} s^{-1} in 250-mL conical flask shaken at 120 rpm in an orbital shaker. Experiments were carried out from cells collected at the mid-log phase of growth.

Cell number and Cell density

Cell number and cell density were measured every 24 hours up to 10 days. Haemocytometer was used to calculate the number of cells. Cell density was measured by UV-Visible spectrophotometer (Thermo scientific 210 India) by taking the optical density at 600 nm.

Biomass measurement

To measure algal biomass, dry weight (W g/L) was calculated gravimetrically as follows:

W= (W2-W1) × 1000/V

Where W1 is the weight of the empty falcon tube (g), W2 is the weight of dried microalgae and the tube, and V is the culture volume.

Determination of Protein content

5 ml of algal culture was pellet down by centrifugation and pellet was suspended in NaOH (1 M) and boiled. By using Lowry method[6] protein present in the sample was estimated.

Determination of Lipid content

The total lipid content was measured by following the modified gravimetric method[7]. The lipid content was calculated by the following equation.

Y (%) = WL-WP/DCW×100

Y= lipid content, WL= weight of lipid, WP= pre-weight of the vial, DCW= dry cell weight

Determination of Carbohydrate content

The carbohydrate content of the algae was determined using the Anthrone reagent method[8].

Determination of pigment composition

Total chlorophyll and carotenoids were extracted and measured[9].

Chl A (μg/ml) = (12.7×A663) -(2.69×A645)

Chl B (μg/ml) = (22.9×A645)-(4.68×A663)

Total chlorophyll (μg/ml) = Chl A+ Chl B

Carotenoids (μg/ml) = (1000×A470-1.63chla -104.9 chlb)/221

RESULTS AND DISCUSSION

The biochemical composition of 6 algal species measured at the end of the batch culture experiment is shown in Table 1. *S. acutus* showed 52.29% of protein content followed by *C. vulgaris* (51.45%) (Table 1). The *C. reinhardtii and D. armatus* displayed the lowest protein content

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of 45.21% and 45.45% of dry weight. *C. reinhardtii* (48%), *C. vulgaris* (51-58%), *and C. pyrenoidosa* (57%), and *S. obliquus* (50-56%) had an average protein content were reported[10]. In *Chlorella* (51-58%) *Scenedesmus* (50-56%), and *Chlamydomonas* (43-56%) respectively were reported[11].

Carbohydrate percentage was significantly affected by the species type. *C. pyrenoidosa* and *C. sorokiniana* had the highest carbohydrate of about 22.88% and 19.78%, while *S. acutus* (12.70%) and *C. vulgaris* (14.73%) had the lowest (Table 1). The average carbohydrate composition of dry algal biomass basis in *C. reinhardtii*, *C. vulgaris*, *C. pyrenoidosa*, and *S. obliquus* was 17%, 12-17%, 26%, and 10-17%, were reported[10]. The carbohydrate content in *Chlorella*, *Scenedesmus*, and *Chlamydomonas* was reported as 12-17%, 10-52%, and 2.9-17% were reported[11].

The highest lipid content (22.91% of DW) was noted in *C. sorokiniana* ($p<0.05$) followed by *C. pyrenoidosa* (21.69%), and the lowest content was found in *S. acutus* (15.14%) and *C. reinhardtii* (18.49%) (Table 1).

The fatty acids found in algal species can be either saturated or unsaturated, with carbon numbers ranging from C12 to C22. Due to high differences in chlorophyll content between cultures and conditions, no major differences in total lipid content were observed in *C. vulgaris,* which ranged from 14.3 to 18.4% of dry weight in all cultures[12].

No specific strain of algae species can be considered the best in terms of oil yield for biodiesel production[10]. The *C. reinhardtii*, *C. vulgaris*, *C. pyrenoidosa*, and *S. obliquus*, respectively, had average lipid content of 2%, 14-22%, and 12-14% of dry algal biomass[10]. The 14-22%, 12-14%, and 14-22% lipid content of *Chlorella sp*., *Scenedesmus sp*., and *Chlamydomonas sp*. was reported[11].

C. vulgaris shows highest total chlorophyll content (23.35±0.74 g/ml), followed by *C. reinhardtii* (22.33±0.90 g/ml) but, *S. acutus* showed the lowest (16.77±0.72 g/ml). *D. armatus* (1.46±0.32 g/ml), *C. pyrenoidosa* (1.28±0.28 g/ml), and *C. reinhardtii* (1.23±0.27 g/ml) is the species with the highest overall carotenoid content, while *S. acutus* (0.98±0.21 g/ml) has the lowest (Table 2). Under dark conditions, *C. vulgaris* cultures had the highest chlorophyll content, 9.16 mg/g, which increased to 14.06 mg/g in the presence of light[12].

Figure 1 shows the cell count and cell density of 6 microalgae from day 1-10. *C. vulgaris* (6.64±0.04 cells/ml) and *C. sorokiniana* (5.91±0.35 cells/ml) had the highest cell number values, while *C. pyrenoidosa* (4.72±0.09 cells/ml) had the lowest cell count (Figure 1).

C. vulgaris had the highest cell density of 1.30±0.05, followed by C. *sorokiniana* (1.27±0.06) and *D. armatus* (1.27±0.03). The *C. pyrenoidosa* (1.10±0.01) and *S. acutus* (1.13±0.01) had the lowest cell density (Figure 1). The results of the cell count and cell density experiments confirmed that these two variables are nearly correlated. Under autotrophic conditions, it was critical to generate the most biomass for biofuel production. Figure 1 illustrates the biomass growth trends for all algal species at 0.2, 0.4, 0.6, 0.8, and 1.0 optical density, demonstrating that biomass increased over time in all species until day 10. The highest biomass was noted in *C. sorokiniana* (1.02±0.02) and *C. vulgaris* (0.87±0.02) and the lowest was observed in *S. acutus* (0.79±0.05).

CONCLUSION

The macromolecule contents of the studied 6 microalgae species are varied significantly in accordance with species type with few exceptions. Furthermore, the biochemical composition of microalgae differs between species and is also affected by environmental and growth conditions. Microalgal lipids are a source of safe and sustainable biofuels that have gotten a lot of attention because of their ability to produce biodiesel and hydrocarbons. Several researchers believed that biodiesel extracted from microalgae could eventually replace petroleum-derived fuels, but low lipid content in algae and high-cost oil harvesting technologies have slowed the commercialization of microalgal biodiesel.

Conflict of interest: None

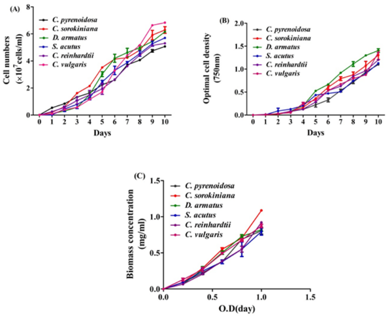

Figure 1: Cell density and cell number and biomass of microalgae under different growth parameters. Size-dependent distribution of cell and the correlation of optical density (A) cell number (B) Cell density (C) Biomass. Each value represents the mean of triplicates

Table-1: Protein, Lipid, and carbohydrate composition of algae on a dry matter basis (%). Total chlorophyll and carotenoid (µg/ml) profile of six microalgae. Each value represents the mean of triplicates

Algal species	Protein (%)	Lipid (%)	Carbohydrates (%)	T-Chl (µg/ml)	Carotenoids (µg/ml)
C. reinhardtii	45.21	18.49	16.24	22.33 ± 0.90	1.23 ± 0.27
C. sorokiniana	48.44	22.91	19.78	18.66 ± 0.74	1.01 ± 0.22
C. vulgaris	51.45	19.37	14.73	23.35 ± 0.74	1.02 ± 0.22
C. pyrenoidosa	48.18	21.69	22.88	18.48 ± 0.93	1.28 ± 0.28
S. acutus	52.29	15.14	12.70	16.77 ± 0.72	0.98 ± 0.21
D. armatus	45.45	20.19	16.58	19.26 ± 1.07	1.46 ± 0.32

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