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# Biomass composition of Spirulina platensis, Scenedesmus dimorphus and Chlorella vulgaris; a comparative study

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**Abstract:** Microalgae are considered a very important source for numerous applications in different fields such as biofuel, food, animal feed, pharmaceutical and biofertilizer owing to their important biochemical composition. Accordingly, three different microalgae species were isolated, identified and their growth on different standard nutrient media was evaluated. The modified *Navicula* nutrient medium supported relatively the highest growth of all the tested microalgae. The test microalgae maintained different specific growth rates (µ) and growth doublings per day ($Dd^{-1}$). The dried algal biomass was used for protein, carbohydrate and lipid analyses. *Spirulina platensis* maintained relatively the higher protein content (57.55%), *Scenedesmus dimorphus* maintained relatively the higher carbohydrate content (29.6%), while *Chlorella vulgaris* maintained relatively the higher lipid content (10.8%). The frozen algal biomass was used to determine the phytohormones content for all the tested microalgae. *Scenedesmus dimorphus* showed the highest content of Cytokinin (24.11 µg $100mL^{-1}$), IAA (63.54 µg $100mL^{-1}$) ABA (4.29 µg $100mL^{-1}$), while *Spirulina platensis* maintained the highest content of GA3 (130.054 µg $100mL^{-1}$). This study may provide a strong recommendation for the promising use of *Spirulina platensis*, *Scenedesmus dimorphus* and *Chlorella vulgaris* in the production of biofertilizers.

**keywords**: Microalgae, Isolated microalgae, Scenedesmus dimorphus

## 1.Introduction

Microalgae are prokaryotic or eukaryotic photosynthetic microscopic organisms found in all the aquatic systems such as, freshwater, seawater, hypersaline lakes and even in deserts and arctic ecosystems [30]. They serve as sunlight-driven cell factories that convert carbon dioxide ($CO_2$) into raw materials for producing biofuels (e.g., biohydrogen, biodiesel and bioethanol), animal food chemical feedstocks and high-value bioactive compounds [43, 22, 34].

They have much higher growth rates and productivity when compared to agricultural crops and other aquatic plants, producing biomass that are rich in many biologically active compounds like lipids, proteins, polysaccharides, enzymes, phytohormones, vitamins, sterols, and other high-value compounds with pharmaceutical and nutritional importance that can be used commercially[12,28].

Numerous species of microalga are rich with protein content with high concentrations that range from 42% to over 70% in certain cyanobacterial species such as *Spirulina platensis* [23,26] and (42–58%) of biomass dry weight in green algae such as *Chlorella vulgaris* [4,24,36,38,39], these microalgae represent an ideal source of nutrients or functional foods for both human and animals [7].

In addition to protein biosynthesis microalgae synthesized glucose and starch-like energy storage products inside chloroplast [47]. The carbohydrate percentage varies among microalgal species depending on cultivation and environmental conditions for example *Spirogyra sp*. (33–64%), *Porphyridium cruentum* (40–57%), *Chlorella emersonii* (37.9%), *Chlorogloeopsis fritschii* (37.8%) [8, 28].

Among the biochemical components of microalgal biomass, they contain lipids as a main components between 20% to 50% of their dry biomass (w/w), that can varies depending on species and growth conditions [43]. Under environmental stress conditions like phosphate or nitrogen starvation, triacylglycerols are accumulated in microalgae biomass that are useful for biodiesl production [6, 13,18].

In addition to protein, lipid and carbohydrate, microalgae biomass contain phytohormones that act as growth regulators for plants affecting crop yields and maintainance to abiotic and biotic stress factors [28].

Based on these information, the present study aimed at investigating the growth potential of three microalgae namely *Chlorella vulgaris*, *Scenedesmus dimorphus* and *Spirulina platensis* on different standard nutrient growth media to select the medium that will support the highest biomass production. Biomass content of protein, carbohydrate, lipid and phytohormones will be analyzed to evaluate the biomass potential as plant biofertilizer

## 2.Materials and Methods

### Isolation and identification of microalgae

Fresh water samples were collected from the River Nile at Delta region in the front of Mansoura University **[**31] and centrifuged at 4000 rpm for 10 minutes then supernatant was discarded and the precipitated plankton pellets were picked up by sterile needle and streaked on Bold Basal medium (BBM) [9] solidified with 1.5% (w/v) of bacteriological agar. The plates were then incubated for two weeks at 25 ± 2 $^{o}$C under continuous light of 12.829 µ mol $m^{-2}$ $s^{-1}$. A simple stereomicroscope was then used to examine and locate microalgae colonies growing on the surface of agar plates and the individual colonies were picked up by sterile needle, restreaked on the agar plates of BBM [9] for more purification and identification.

These procedures result in the isolation of three unialgal isolates that were identified according to [2,21,27,20,37]. Classification and nomenclature of the isolated microalga were checked against the recent information posted at the webpage of alga base (http://www.algaebase.org ).

### Isolated microalgae

The isolated microalgae include two species of green algae *Chlorella vulgaris* Beyerinck, *Scenedesmus dimorphus* (Turpin) Kützing and one species of cyanophyceae *Spirulina platensis* var. *tenuis* C.B.Rao.

### Effect of different nutrient media on growth of the isolated microalgae

The growth of test microalgae was assessed using three different standard nutrient media including, modified *Navicula* nutrient medium [45] BG11 medium [44] and Bold Basal medium [9]. The started inoculum of all tested microalgae was equivalent to 0.04 g $L^{-1}$ dry wt. . Cultures were incubated for two weeks under continues illumination of 45 µmol $m^{-2}$ $s^{-1}$ at 25 ± 2$^{o}$ C, pH = 7 for *Chlorella vulgaris* and *Scenedesmus dimorphus* and at 35 ± 2$^{o}$ C, pH = 9 for *Spirulina platensis*.

### Growth assessment

### Cell count

The algal growth was estimated by direct cell count using standard haemocytometer technique [16].

### Dry weight

Gravimetric determination of dry weight was carried out by centrifugation a suitable volume of algal culture. The supernatant was carefully, decanted, the sediment algal cells were washed with distilled water, re-centrifuged, transferred to a dry and pre-weighted crucibles, dried in a hot air oven at 105$^{0}$C, cooled down in a desiccator for one hour, and then reweighted to obtain the average dry weight (g $l^{-1}$) of the tested algae [16].

### Growth rates

The growth rates of the algal cultures were calculated according to [17**]** using the following equations:

$$\text{Growth rate; } \mu = \frac{\ell n\ (N / N_o)}{dt}$$

Where $N_0$ is the initial cell density (cell $ml^{-1}$) and N is the cell density at a given time t. Doubling per day rate ($Dd^{-1}$) was calculated as follows:

$$\text{Doubling per day; } Dd^{-1} = \frac{\mu}{\ell n 2}$$

**Biochemical properties:**

**Total protein content**

Crude protein content was analyzed by the method of **[**11] and modified by [46].

**Total carbohydrate content**

Carbohydrate content was determined according to [19].

**Total lipid content**

Lipid extraction followed the exhaustive Soxhlet method [35].

**Phytohormones content**

Frozen samples of the test microalgal species were sent to Arid Land Agricultural Research and services Center Faculty of Agriculture –Ain Shams University for phytohormone analysis auxins (IAA), gibberellins ($GA_3$)**,** cytokinin (CK), abscisic acid (ABA) by gas liquid chromatography (GLC) according to [41].

**3. Results and Discussion**

**Growth of different microalgae.**

**Effect of different nutrient medium**

Table 1 illustrates the growth (expressed as gram dry weight per liter) of different tested microalgae in three different nutrient growth media. It is obvious that the modified *Navicula* medium supported relatively the highest growth of all tested microalgae, the cyanobacterium *Spirulina platensis* maintained the highest growth production (0.234±0.012 $gL^{-1}$) followed by *Scenedesmus dimorphus* (0.165±0.008 g $L^{-1}$), *Chlorella vulgaris* (0.147±0.007 g $L^{-1}$), respectively.

**Growth assessment of different tested microalgae on modified *Navicula* medium**

Growth curves of *Chlorella vulgaris* and *Scenedesmus dimorphus* were plotted using cell count as illustrated in Figure 1, while growth curve of *Spirullina platensis was* plotted using average dry weight as illustrated in Figure 2. At the end of rhe sixth day *Chlorella vulgaris* and *Scenedesmus dimorphus* maintained the highest growth ($245\pm12.25^{*}10^{6}$ cells $L^{-1}$), ($161\pm8.05^{*}10^{6}$ cells $L^{-1}$) respectively. While at fifteenth day *Spirullina platensis* maintained the maximum biomass production (1.207±0.06 $gL^{-1}$).

**Growth rates**

In an attempt to get an accurate growth comparison of different tested microalgae both specific growth rate (μ) and growth doubling per day ($Dd^{-1}$) were calculated. The results are listed in Table 2.

It is evident that different tested microalgae exhibit different specific growth rates and growth doubling .The highest specific growth rate and growth doubling were recorded for *Chlorella vulgaris* (0.64 ±0.032), and (0.83 ±0.041) respectively, while the lowest were recorded for *Spirullina platensis* (0.178±0.009),and (0.257± 0.013), respectively.

**Biochemical composition of the tested microalgae**

**Total lipid content, total protein content and total carbohydrate content**

Weight percentage of total lipid, total protein and total carbohydrate of the tested microalgae were shown in Figure 3. The highest percent of total lipid was recorded for *Chlorella vulgaris* (10.8±0.54%) and the lowest were recorded for *Scenedesmus dimorphus* (3.8 ±0.19%). *Spirullina platensis* maintained relatively the highest protein content (57.55±2.87%) compared with *Chlorella vulgaris* and *Scenedesmus dimorphus* (55.25±2.7%), (32.17±1.6 %), respectively. In the other side *Scenedesmus dimorphus* maintained the highest percentage of total carbohydrate content (29.6±1.48%) compared to *Spirullina platensis* and *Chlorella vulgaris* (25.12±1.25%), (22.7±1.13%), respectively.

**Phytohormones content**

Phytohormone content was assessed at the end of growth (stationary phase) of each microalga. The results were listed in Table 3 shown that phytohormone content was species dependent. For instance *Scenedesmus dimorphus* maintained relatively high content of cytokinin (Benzyl, Kinitin, Ziaten), auxins (IAA), abscisic acid (ABA)(24.11 µg $100mL^{-1}$, 63.54 µg $100mL^{-1}$, 4.29 µg $100mL^{-1}$), respectively. While *Spirullina platensis* maintained the highest content of gibberellins ($GA_3$) (130.054 µg $100mL^{-1}$).

**Discussion**

The experimental results (Table 1) indicated that the modified *Navicula* nutrient medium supported the highest growth of the all tested

microalgae. It has been well documented [10, 14, 29] that the culture medium not only affects the microalgae growth but also affects their metabolic activities. The marked increase in dry weight of all tested microalgae, grown in the modified *Navicula* medium, clearly indicated that this medium is the most favourable growth medium for all the tested microalgae.

The tested microalgae maintained significantly ($P \leq 0.05$) different specific growth rates (μ) and growth doubling per day ($Dd^{-1}$), when grown on modified *Navicula* medium (Table 2). This is expected and widely reported that growth rate is largely species dependent parameter [25].

Since the composition of nutrient media affects not only biomass production but also the composition and yield of microalgae metabolites. It seems logic to analysis the biochemical composition of the tested microalgae grown on modified *Navicula* nutrient media, that supported the highest biomass production compared to other media.

The results listed in Figure 3 illustrated that *Spirulina platensis* and *Chlorella vulgaris* maintained the highest protein content, these results are in agreement with results obtained by [ 5,28]. It is relevant to mention that the high protein content of various microalgae species is one of the main reasons to consider them as a potential unconventional source of protein [42]. It has been widely known that different species of cyanobacteria and in particular *Spirulina* are rich with proteins which can amount to up to 70 % of their dry weight biomass under optimum growth conditions, making them a superior source of proteins, compared to conventional plants like Soybean and thus they are consumed as human food [33].

The high percent of carbohydrate content recorded for *Scenedesmus dimorphus* (Figure 3) goes in harmony with results obtained by[**3,32**]. It has been reported that microalgal polysaccharides showed good capacity for improving plant growth, offering an interesting potential use as biostimulants[**15]**.

The high lipid content recorded for *Chlorella vulgaris* (Figure 3) may trigger to further research to investigate the lipid profile of this algae to evaluate its suitability as a renewable bioresource for biodiesel production. It has reported that most microalgae are rich in polar lipids in the exponential phase of growth, and they accumulate triacylglycerols under stress conditions and PUFAs which are essential for the nutrition of humans and aquatic animals [32, 40].

The results of phytohormones content were shown in Table 3. The high content of cytokinin, auxins (IAA), abscisic acid (ABA) recorded for *Scenedesmus dimorphus* biomass and the high content of gibberellins ($GA_3$) recorded for *Spirullina platensis* biomass may hold a promising role for the future of such alga in biofertilizer production. It has been widely known that cytokinins enhance cell division, regulate shoot, root development, stimulate leaf growth in addition to flower, fruit, and seed formation. Cytokinins also stabilize photosynthetic machinery, suppress senescence, enhance sink strength and nitrogen acquisition. Auxins promote root elongation strongly. Abscisic acid is a plant hormone, whose formation increases with water stress to induce adaptive stress responses. Gibberellins control developmental processes such as germination, shoot elongation, tuber formation and flowering. [1].

Finally, it can be concluded that *Chlorella vulgaris*, *Spirulina platensis* and *Scenedesmus dimorphus* biomass will be in a focus of further research as renewable resource for the future of biofertilizer production.

**Table 1:** Average dry weight ($gL^{-1}$) of tested microalgae grown for two weeks in different standard growth media under lab favourable growth condition (Biomass±SD)

| **Isolats** | BG11 | Bold basal | Modified *Navicula* |
|---|---|---|---|
| ***Chlorella vulgaris*** | 0.13±0.006 | 0.048±0.002 | 0.147±0.007 |
| ***Scenedesmus dimorphus*** | 0.09±0.004 | 0.049±0.002 | 0.165±0.008 |
| ***Spirullina platensis*** | 0.17±0.009 | 0.039±0.002 | 0.234±0.012 |

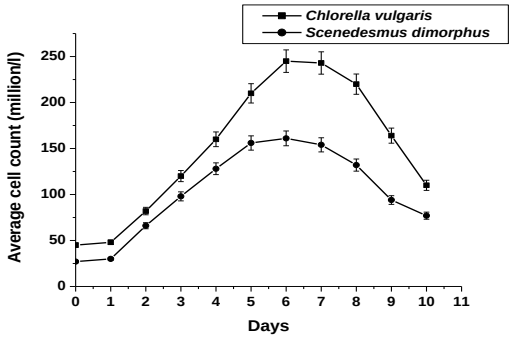


**Figure 1:** Growth curves of *Chlorella vulgaris* and *Scendesmus dimorphus* expressed as ($10^6$ cell $L^{-1}$) grown in modified *Navicula* nutrient medium for two weeks under lab favourable growth condition (Biomass±SD)

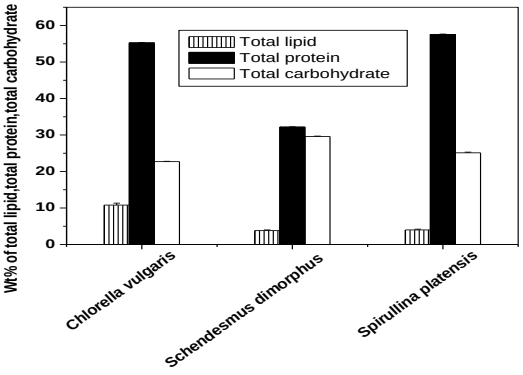


**Figure (3):** Wt % (w/w) of total lipid, total protein and total carbohydrate content of biomass of different tested microalgae grown in modified *Navicula* nutrient medium

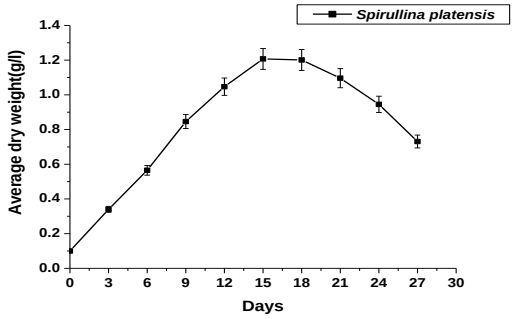


**Figure 2:** Average dry weight of *Spirullina platensis* expressed as ($gL^{-1}$) grown in modified *Navicula* nutrient medium for four weeks under lab favourable growth condition (Biomass±SD)

**Table 2:** Specific growth rate (μ) and growth doubling per day ($Dd^{-1}$) of different tested microalgae grown in the modified *Navicula* nutrient medium.

| **Microalgae species** | **μ** | **$Dd^{-1}$** |
|---|---|---|
| Chlorella vulgaris | 0.64 ±0.032 | 0.93 ±0.046 |
| Scenedesmus dimorphus | 0.58±0.029 | 0.83 ±0.041 |
| Spirullina platensis | 0.178±0.009 | 0.257±0.013 |

**Table 3:** Variation in phytohormones content (benzyl, kineten, ziaten, GA3, IAA, ABA) of different tested microalgae grown in modified *Navicula* nutrient medium.

| **Microalgae isolates** | **Phytohormones content ( μg $100mL^{-1}$)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Benzyl** | **Kineten** | **Ziaten** | **Cytokinin** | **GA3** | **IAA** | **ABA** |
| *Chlorella vulgaris* | 1.76 | 1.29 | 16.94 | 19.99 | 56.70 | 58.08 | 3.79 |
| *Scenedesmus dimorphus* | 6.04 | 5.53 | 12.54 | 24.11 | 55.74 | 63.54 | 4.29 |
| *Spirullina platensis* | 1.48 | 1.16 | 10.4 | 13.04 | 130.054 | 14.81 | 0.981 |

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