Evaluation of Porphyridium purpureum and Nannochloropsis sp. for Carbohydrates and Lipids Production

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This study was aimed to evaluate the growth conditions of Porphyridium purpureum and Nannochloropsis sp. microalgae for carbohydrates and lipids productivity increase. The cultivation experiments for both microalgae strains were done in triplicates using the artificial seawater (ASW) as culture media. The lipids were separated from the algal biomass and the exo-polysaccharides content from the supernatant. The results showed that the highest carbohydrate content is found in Porphyridium purpureum while the highest lipid content was found in biomass.

Keywords: microalgae, polysaccharides, lipid, Porphyridium purpureum, Nannochloropsis sp.

Renewable liquid biofuels carbon-neutral are needed to partially replace petroleum-derived fuels because they have a negligible contribution to climate change and can be continuously regenerated, while the conventional fuels are depleting nonstop [1]. New, renewable energy sources, such as microalgae may become useful raw-materials in biofuels and biorefinery, due to their many advantages, including sustainable growth, high photosynthetic efficiency, areal productivity and oil content; also, they have the potential advantage of being harvested daily. Moreover, they do not compete with agricultural areas, and sources of potable water [2].

The screening of microalgal candidates for lipid and/or carbohydrates and lipids productivity increase. The cultivation experiments for both microalgae strains were done in triplicates using the artificial seawater (ASW) as culture media. The lipids were separated from the algal biomass and the exo-polysaccharides content from the supernatant. The results showed that the highest carbohydrate content is found in Porphyridium purpureum while the highest lipid content was found in biomass.

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collection of algal strains of University Babes Bolyai, Cluj Napoca, Romania. Both strains were grown using artificial seawater (ASW) as culture medium with the following composition: 15.0g/L NaCl; 3.05g/L MgSO₄·6H₂O; 2.8g/L MgCl₂·6H₂O; 0.75g/L CaCl₂·2H₂O; 1.0g/L KNO₃; 0.08g/L KH₂PO₄; 0.54g/L NaHCO₃ (sterilized by autoclavation) and 1 mL/L of trace metal solution: 2.8 g/L H₃BO₃, 2.03 g/L MnSO₄· 4H₂O, 0.222 g/L ZnSO₄·7H₂O, 0.018 g/L MoO₃ (85%), 0.079 g/L CuSO₄·5H₂O and 0.494 g/L Co(NO)₃·6H₂O, previously mixed with 1 mL/L of chelated iron solution (0.69 g FeSO₄·7H₂O + 0.93 g Na₂EDTA in 80 mL demineralized water) sterilized by filtration with 0.2mm PTFE filter [12].

**Growth Measurements**

The algal biomass cultivation tests were performed in 100 ml aerated bottles adding 2 mL of inoculum to 50 mL of sterilized culture medium. The flasks were supplemented with 0.05 mL/L of the micronutrient solution and 0.05 mL/L of chelated iron solution. The cultivation experiments for both microalgae strains were done in triplicates using an orbital shaker-Incubator ES-80 which provide an agitation of 100 rpm, at environmental temperature and light intensity of approximately 18W/30. When the cultures reached the end of cultivation time, biomass was separated from the culture medium by centrifugation at 10000 rpm for 20 min, using a Hettich Rotina 380R centrifuge.

**Lipid extraction**

The algal lipid fraction was extracted and characterized by the method detailed in Galan et. al., [17]

**Polysaccharides extraction**

The exo-polysaccharides content from the supernatant obtained after centrifugation of Porphyridium purpureum biomass was extracted following the protocol detailed in Patel et.al. [13] using 99% Ethanol with ratio 1:1 EthOH : Supernatant in a 50 mL tube. Afterwards the tube was shaken for 5 minutes and stored in at -20 °C for 12 h. After storage the sample was centrifuged at 5000 rpm for 10 min [18]. Carbohydrate content from algal biomass was quantified as glucose equivalent according to phenol-sulfuric acid method [19].

**Results and discussions**

### Growth measurements

Both strains were grown using ASW as culture medium. Data refer to cellular concentration and optical density, both parameters are shown in Table 1. As can be seen, after 10 days of cultivation, Nannochloropsis sp. reached the highest cellular concentration (3.413×10⁶ cell/ml) and optical density (0.73±0.08), while Porphyridium purpureum exhibited the lowest cellular concentration (1.206×10⁶ cell/mL) and optical density (0.72±0.09). Regarding biomass production, for Porphyridium purpureum were obtained 1.50±0.01 g/L, an almost double value compared to Nannochloropsis sp., 0.71±0.02 g/L. However, the highest biomass productivity was observed for Porphyridium purpureum strain, despite its lower optic density and cellular concentration values. Cell morphology image 40x of the two strains studied is presented in figure 1. In both cases, solitary or small clusters cell with smooth outer surface of plasma membrane were observed.

**Total polysaccharides content**

The polysaccharide content is an intermediate reserve in some algae under chemical and physical stress. Carbohydrates tend to accumulate in the algae grow deprived of nutrients or light. The highest cell carbohydrate content of 13.20±0.05 % was observed for Porphyridium purpureum, followed by Nannochloropsis sp., with 9.15 ±0.02% (Table 2). Under stress conditions Porphyridium purpureum strain has the ability to excrete in the culture medium exo-polysaccharide. In this study, the concentration of exo-polysaccharide extracted from culture media by precipitation with ethanol was 1.43±0.25 g/L. These compounds serve as antiviral agents, health foods, antioxidants, they have anti-inflammatory properties and, also, a role in the immunomodulatory system; they

**Table 1**

| Algae Strain          | Nannochloropsis sp. | Porphyridium purpureum |
|-----------------------|---------------------|------------------------|
| Cellular concentration, cell/mL | 3.413×10⁶          | 1.206×10⁶              |
| Optical density       | 0.73±0.08 (at 540 nm) | 0.72±0.09 (at 750 nm)  |
| Dry Biomass, g/L      | 0.71±0.02           | 1.5±0.01               |

**Table 2**

| Strain              | Nannochloropsis sp. | Porphyridium purpureum |
|---------------------|---------------------|------------------------|
| Exo. polysaccharides, g/L | 0                  | 1.43±0.25              |
| Carbohydrates from biomass (%) | 9.15±0.02           | 13.20±0.05             |
| Lipids (%)          | 52.07±0.07          | 46.77±0.09             |

Fig. 1. Cells morphology image 40x of Nannochloropsis sp. (A) and Porphyridium purpureum (B)

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may also be used as lubricants for bone joints, or even as drag-reducing substances for ships [20-23].

Lipid and fatty acid profiles

The lipid content of the algal biomass was determined by thermogravimetric analysis. The lipids percent shown in Table 2 is composed of neutral lipid, glycolipid and phospholipid. The recorded thermogravimetric analyzes for the Porphyridium purpureum strain (figure 2) and Nannochloropsis sp., (figure 3) have the same decomposition profile. In the first step, at temperatures up to 100 °C, the weight loss of the sample was very low. This slight decrease in sample weight is mainly due to the evaporation of moisture. From 100 to 250 °C, the loss of weight is due to evaporation of the volatile compounds present in the biomass sample. The maximum decomposition level of Porphyridium purpureum biomass was observed in the temperature range from 250 to 280 °C, indicating loss of the lipid fraction, while for the Nannochloropsis sp. algal biomass, the degradation step corresponding to the lipid fraction was recorded in the temperature range from 297 to 305°C. This small temperature difference can be explained by the different composition of the fatty acids present in the two algal oils, especially the saturated fatty acid content. Algal oil from Nannochloropsis sp. has a saturated fatty acid content of 43.26±0.41 %, and the Porphyridium purpureum algal oil has a percentage of 33.21±0.33%. The highest lipid content was observed in Nannochloropsis sp. biomass followed by Porphyridium purpureum.

The fatty acid distribution of algal oils extracted from the two algal strains is shown in Table 3. The highest content of saturated fatty acids is in the Nannochloropsis sp. (43.26±0.41%) oil, followed by Porphyridium purpureum (33.21±0.33%). With regard to the monounsaturated fatty acid content from the two algal oils analyzed, again Nannochloropsis sp. has the highest content of 33.6±0.35% and Porphyridium purpureum of 6.8±0.21%. Regarding the content of polyunsaturated fatty acids, the algal oil extracted from the strain Porphyridium purpureum has 58.2±0.43%, while that of Nannochloropsis sp. has 23.68±0.18%.

Table 3

| Fatty acids content | Porphyridium purpureum | Nannochloropsis sp. |
|---------------------|------------------------|---------------------|
| Saturated fatty acids, % | 43.26±0.41% | 33.21±0.33% |
| Monounsaturated fatty acids, % | 33.6±0.35% | 6.8±0.21% |
| Polyunsaturated fatty acids, % | 58.2±0.43% | 23.68±0.18% |

Conclusions

The aim of this work was to evaluate the potential of green microalgae Nannochloropsis sp. and red microalgae Porphyridium purpureum for carbohydrates and lipids production. The both strains were growth using artificial sea water as culture medium. The lipid and carbohydrate content of the algal biomass was determined by thermogravimetric analysis. The results showed that the two microalgae strains studied were capable to produce carbohydrates and lipids. The highest carbohydrate content of algal biomass was observed for Porphyridium purpureum, meanwhile the highest lipid content was determinate in Nannochloropsis sp. biomass. The fatty acid distribution of algal oils extracted from the two algal strains indicated that Nannochloropsis sp. oil has a saturated fatty acid content of 43.26±0.41%, and the Porphyridium purpureum algal oil has a content of polyunsaturated fatty acids of 58.2±0.43%.

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