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Influence of SHF Treatment on Lipid Output from Microalga *Chlorella Sorokiniana*

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Abstract. This work is devoted to investigation of methods of obtaining and usage of lipid fraction from microalgae. We describe methods for obtaining lipid fractions from microalgae *Chlorella Sorokiniana* by means of Soxhlet apparatus. We studied influence of microalga biomass drying technique (IR and lyophilization) and cell disintegration technique on lipid output from biomass. We investigated physical (SHF-radiation) and mechanical (grinding) treatments aiming at cell disintegration. It was shown, that maximum lipid output is obtained at combined treatment of biomass by lyophilization and SHF-irradiation. When comparing total lipids output from lyophilized biomass without and after disintegration (by mechanical and physical treatments), we didn't notice any notable difference. Lyophilic treatment simultaneously dehydrates biomass and results in maximum cell disintegration, which provides maximum lipids output without additional disintegration stage (13.2%). It was proved that biomass lyophilization results in maximum lipids output.

1. Introduction

Microalgae utilizations are broad and diverse. Having rich vitamin and protein content, *Chlorella* is used as a livestock feed, as well as for human consumption. Microalga implementation for medical and ecological purposes is now under investigation [1]–[4]. An interesting research area is obtaining of lipids from microalgae, which can be used as sources of Omega-3, Omega-6 or biofuel, depending on its' fatty acid content. Methods of lipids obtaining from algae have been studied worldwide.

American scientists [5] have studied 3 species of *C. sorokiniana* strain, and developed a technique for lipid extraction. Algerian scientists [6] have grown *Chlorella* for further biofuel obtaining. The highest amount of fatty acids is obtained upon condition that nutrition media contains increased amount of nitrogen. Consequently, the obtained biofuel is of higher quality.

The authors [7] have obtained biofuel from *C. vulgaris* for diesel engines in laboratory conditions. The investigations have shown that BG-11 medium is the most efficient for the studied *Chlorella* types. The authors [8] have investigated influence of temperature and nutrition broth composition on protein and lipids production for 4 strains of *Chlorella*. Microalgae were cultivated at non-laboratory conditions using GWP-II photobioreactor. It was established that these strains are able to develop in medium, prepared basing on sea water at rather high temperature. The paper [9] presents data on influence of temperature and medium composition on *C. Sorokiniana* lipid synthesis. It was shown, that this microorganism is able to sustain temperature up to 42 °C and has maximum growth rate at 37



°C. Nowadays it is known that many other algae species may be valuable sources of biofuel [10], [11]. Thus, for example, cultivation of *C. marina* и *Skeletonema costatum* in laboratory conditions and in large amounts (100 l) has shown high productivity of lipids and fatty acids: palmitinic (16:0), oleinic (18:1), linoleic (18:2) [12].

For obtaining maximum lipid output one should choose not only conditions for directed cultivation, but also method for disintegration of alga cell wall aiming at providing access of solvent into internal cell space at lipid extraction stage [13]–[15]. Physical treatment means action of physical field (hydrodynamic, acoustic, etc.) on cell, which leads to destruction of cell wall.

Since 2017 research works on cultivation methods of *Chlorella Sorokiniana* microalga for further obtaining of valuable components, including lipids, are conducted at Peter the Great St. Petersburg Polytechnic University [1]–[4].

The aim of this work is investigation of influence of disintegration method of *Chlorella Sorokiniana* microalga dry biomass on lipids output and determination of fatty acid content of the obtained fraction.

2. Materials and methods

Chlorella Sorokiniana microalga growth was carried out in a closed system, photobioreactor, which significantly decreased risk of contamination from outer environment [20]. Cultivation is carried out in nutrient broth containing macro- and microelements [15–16]. Biomass gathering is performed on the 10 day of cultivation. For this purpose cell sedimentation takes place for 3 days in cylinders (height 50 cm, diameter 5 cm). The precipitate is centrifuged with 6000 rpm rate for 10 minutes. Dewatered biomass is dried using IR-dryer and lyophilization.

For IR-drying we used IR-lamp, maximum biomass heating was $T = 37^\circ \text{C}$. Drying was carried out up to constant weight. Drying duration was 12 hours. For sample lyophilization we used lyophilic drying installation 10 N.

For total extraction of lipid fraction we carried out cell disintegration using: physical approach (SHF-irradiation); mechanical approach (grinding).

For physical cell disintegration after lyophilic and IR-drying we added hexane $V = 10 \text{ ml}$ and exposed to SHF-irradiation (power 700 W, frequency 380 MHz at various irradiation time). For mechanical cell disintegration after lyophilic and IR-drying we added hexane $V = 10 \text{ ml}$ and ground it in a porcelain mortar to obtain homogeneous suspension. The obtained suspension and dry biomass of *Chlorella Sorokiniana* microalga were extracted using Soxhlet apparatus (model Büchi E-812 SOX). For this purpose 3 g of dry or wet with hexane after disintegration biomass was put into a cellulose glass (glass dimensions are 33 mm x 94 mm). The solvent system was ethanol: n-hexane (1:9). We used 100 ml of extracting agent for 3 g of dry biomass.

We have discovered that for total extraction of lipids it is necessary to conduct 15 extraction cycles, which takes 3 hours. Extraction process includes 100%-heating of hot plate, 5-minute washing-out at 100%-heating of hot plate and 30-minute dry of extract at 120%-heating of hot plate. The obtained extracts are totally dried out at drying cupboard SNOL at 50°C and then weighted using analytical balance OHAUS RV 214.

Total lipids mass was determined according to the formula:

$$m_{\text{tot}} = m_1 - m_0,$$

Where m_{tot} is lipid extract mass (g); m_1 is glass mass after extraction (g); m_0 is glass mass before extraction (g).

Percentage output of extracted lipids was determined using the formula:

$$M = m_{\text{tot}} \times 100/m,$$

where M is percentage mass of extracted lipids (%), m is dry biomass mass (g).

To maintain the experimental integrity we carried out 3 replicate tests and determined average lipid output (M_{av} , %).

Fatty acid content of the obtained lipids was determined by gas chromatography technique using Agilent 7820AF.

3. Results and discussion

The data on total lipids output depending on way of drying and disintegration (in percents) are presented in Table 1.

Table 1. Output of extracted lipids from dry biomass of *Chlorella Sorokiniana* microalga with and without cell disintegration.

Disintegration technique	Lipids output (M, %) from lyophilized biomass	Lipids output (M, %) from biomass after IR-drying
Without treatment	13.2±0.2	10.4±0.2
SHF (10 s)	13.7±0.2	10.9±0.2
SHF (20 s)	14.9±0.2	11.5±0.2
SHF (30 s)	15.3±0.2	12.8±0.2
SHF (40 s)	15.5±0.2	13.6±0.2
SHF (50 s)	15.5±0.2	13.7±0.2
SHF (60 s)	14.9±0.2	13.0±0.2
Mechanical	13.5±0.2	11.8±0.2

The obtained results (Table 1) show that maximum lipids output is reached after biomass lyophilization and SHF-treatment. At comparison of cell desintegration techniques we have shown, that SHF-irradiation results in maximum output of totallipids. It is clearly seen on the example of biomass after IR-drying.

It is known, that at SHF-irradiation an interaction with polar molecules inside the cell takes place. As a result, molecules, spinning around its' axis, cause significant inter-molecular friction, which leads to boiling of subcellular water and breakage of cell casing. The work [16] shows that SHF irradiation (power 700 W, frequency 280 MHz, time 30 s) increases lipids output from dry biomass of microalgae *Chlorella vulgaris*. Investigation of SHF treatment duration shows that optimum treatment time for all types of drying is 40 s. At shorter time not all cell walls are broken. At longer treatment biomass heating higher than 45 C takes place, which results in lipids oxidation.

When comparing total lipid output from lyophilized biomass without and after disintegration (by mechanical and physical treatments), we didn't notice any notable difference. It is known, that lyophilization, apart from dewatering of biomass, allows one to obtain maximum cell disintegration. This provides maximum lipids output without additional disintegration stage. So, biomass lyophilization is sufficient for further lipids extraction. The obtained lipids fractions might be used as a source for biofuel, which might solve some ecological problems arising at usage of natural fuel and preserve our planet resources.

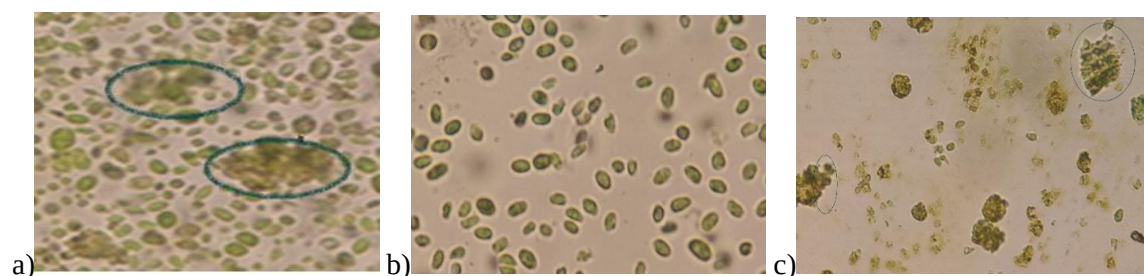


Figure 1. Microphotographs of microalga *Chlorella Sorokiniana* after drying: a) lyophilic; b) IR; c) IR+SHF. Magnification range is 640 times.

4. Conclusions

In this work we studied influence of IR and lyophilic drying of biomass on lipid fraction output. We have shown that maximum lipid output from biomass is provided by lyophilization (13.2%) as compared with IR (10.4%).

It was experimentally shown, that physical cell disintegration of microalgae using SHF irradiation provides maximum lipids output (13.7%). It can be explained by the fact that at IR treatment maximum breakage of cell casing takes place.

5. References

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