BREAKING GREEN ALGAE CHLORELLA SP. CELLS BY APPLYING MECHANICAL TECHNIQUES

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Abstract - Two mechanical techniques (ultra-sonication and ultra-homogenization) were usedin this study for estimation of efficiency of microalgaeChlorella sp. cell breaking. The experiments were performed using non-processed and treated using frost algae suspension (operation duration from 1 to 20 minutes). The highest cell breaking efficiency (50.76 %) was estimated with ultrasonic processor applying treated using frost algae suspension by keeping fixed temperature after 20 minutes.The highest possitive disruptive effect using ultra-homogenizer was 37.55 %. Suspension pre-treatment using frost enables increasing of breaking efficiency till9 %.

Keywords - Algae, Breaking, Ultrasonication, Ultrahomogenization

I. INTRODUCTION

Due to their unique capability to produce and accumulate various beneficials substances under certain conditions, algae such as Chlorella, Scenedesmus, Spirulina, Haematococcus, Dunaliella, Arthrospira, Nannochloropsis, Tetraselmis, Botryococcusand otherhabe been used for many purposes for several years. Since their biomass has a high content of various proteins, carbohydrates, fats, minerals, vitamins, carotenoids, algae are used in the food and feed industry, cosmetics and the production of high-value products rich in polyunsaturated fatty acids (omega-3), pigments, natural antioxidants (e.c. Astaxanthin derived from Haematococcus (Lorenz and Cysewski, 2000))and persistent isotopic biochemicals (Spolaore et al, 2006; Brennan and Owende, 2010). In addition, biomass of some algae species is suitable for production of organic chemicals such as biobutanol and aceton (Harun et al, 2010).

Recently, the growing attention has been focused on research to analyze the potential for the use of algae for biofuel production. Releasing of algae oil as intracellular substance from cells often complicates durable, high resistant to various type of effect walls. Algae usually start to accumulate oil under stress conditions. Being under such conditions is increasing thickness of algae cell walls.

For breaking of algae cells various mechanical (bead beating, ultrasonication, high pressure homogenization, etc) and non-mechanical approaches (physical, chemical, enzymatic) are used (Lee et al, 2012).

The aim of this study is to analyse disruptive efficiency of two mechanical techniques, namely ultra-sonication and ultra-homogenization for cell breaking of green microalgae using pre-treated using frost and non-processed algae suspension.

II. MATERIALS AND METHODS

Green microalgae Chlorella sp. was used for these investigations. Microalgae cells that belong to the Chlorella genus are single, with a spherical or elliptical form, with sizes from app. 2 to 10 μm. The scientists from the State Scientific Institute Nature Research Centre isolated Chlorella sp. from Lithuanian surface waters. Microalgae was cultivated in a laboratory under autotrophic growth condition (growth medium: BG11, growth temperature: 22 ± 2 °C, illumination: ~250 μmol m^{-2} s^{-1}using fluorescent lamps for 10 h daily). Estimation of light intensity was performed using a data logger (model LI-1400) with a LI-190SA Quantum sensor. Microalgae suspension was agitated in order to avoid stagnation and sedimentation of microalgae.

For investigation of algae cells breaking using ultra-sonication mode, VCX 130 ultrasonic processor was used. This ultrasonic processor is a commercial high-frequency vibration transducer (working frequency - 20 kHz, power – 130 W). The experiments were carried out by prolonging operation durations of algae suspension from1, 3, 5, 10 to 20 minutes. In order to achieve the highest efficiency of algae cells breaking using ultrasonic frequency in liquid suspension, the enclosed system was projected and created. This system transfers high frequency to rotating in closed system algae suspension. Peristaltic pump was used to move algae suspension. Liquid speed in the close system was 2000 ml/min.

IKA T25 Ultra TURRAX ultra-homogenizer was used in this study. Microalgae suspensions were treated at different rotor speeds, using 16000, 20000 and 24000 rpm and different durations of suspension treatment (1, 3, 5, 10 and 20 min). The investigations were performed by keeping microalgae suspensions in the graduated laboratory plastic tubes.

To estimate the microalgae cell breaking efficiency, treated using frost (frozen at -18 °C and defrosted)

and non-treated using frost Chlorella sp. microalgae suspensions were used. The efficiency of microalgae cell wall breaking, ECWB (%), was estimated by counting intact and disrupted microalgae cells. For counting of Chlorella sp. cells a light microscope Leica DM500 and a Goriajev chamber were used. Before experiments of cells breaking, algae cells were counted in all grid squares of the Goriajev chamber. Cell averages were found after estimating the algae cell number in each square. After each algae cell disruption experiment, only intact cells were counted in the squares of the chamber.

$$ECWB = \frac{Total\ number\ of\ cells\ before\ experiment - intact\ cells\ number\ during\ experiment}{Total\ number\ of\ cells\ before\ experiment} * 100\ \%$$

All experiments were performed in triplicate, and mean values and the standard deviation were calculated.

III. RESULTS AND DISCUSSION

Efficiency of algae cells wall breaking using ultra-sonication and ultra-homogenization were investigated in this study. In different ways prepared (frozen and defrosted and non-processed using frost) suspensions of Chlorella sp. were used. The experiments were carried out with ultrasonic processor processing microalgae for 1, 3, 5, 10 and 20 minutes. Chlorella sp. cells were started to be broken during the first minutes of using high frequency vibrations (Fig. 1). The minimum number of algae cells (< 7 % for frost-treated algae and < 4 % for non-treated algae) was disrupted using high frequency vibrations with the shortest duration of treatment. The efficiency of cell disruption grows proportionally by increasing the operating duration of the ultrasonic processor. By treating the algae cells with the ultrasonic processor for 20 min, the obtained efficiency of cell disruption for frost-treated algae was almost 50 % and 45.95 % for non-treated algae.

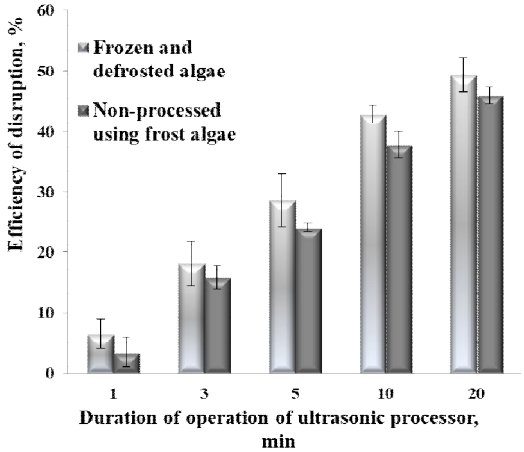


Fig. 1. Change in efficiency of algae cell disruption using ultrasonic processor

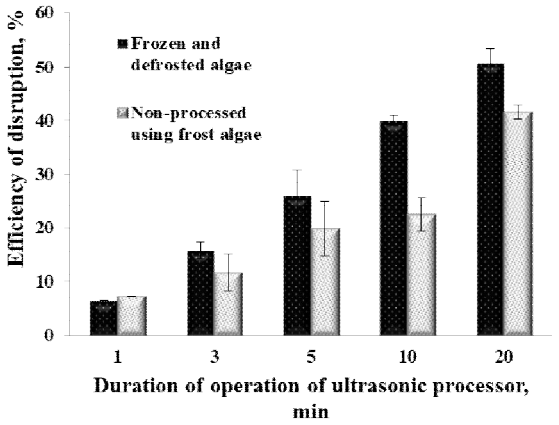


Fig. 2. Change in efficiency of algae cell disruption using ultrasonic processor at a constant temperature of the suspension .

During experiments we were observed that the temperature of suspension increases with an operation time and during the longest operation duration (20 min) temperature of suspension reached app. 90 °C. It has to be noted that high temperature can have negative influence on organic substances being inside of algae cells. Such excess of heat can induce denaturation of proteins, oxidation of mono- and polyunsaturated fatty acids, which may result reduction of oil content(Oehrl et al, 2001).

Temperature can have influence not only on composition and content of substances, but also affect efficiency of algae cell breaking. Taking notice of mentioned above assumptions, we were performed further investigation controlling temperature, i.e. by keeping temperature of algae suspension constant. The results of investigations showed that the temperature does not have a big influence on efficiency of breaking algae cells (Fig. 2). For the frost-treated microalgae Chlorella sp., the results for both cases were approximately the same (49.33 % and 50.76 %, respectively). For the non-treated algae, the efficiency of algae cell disruption was approximately 4 % higher by changing the temperature during high frequency vibrations compared with operating algae suspension at a constant temperature (20 min operation duration).The results of previous study using ultrasonication mode showed similar tendency with microalgae Neochlorisoleabundans. The degree of cell breaking increases by prolonging of operation time till 30 min, however more longer operation duration enhances efficiency very marginally (Wang et al, 2015).Other authors indicated that such disruptive modes as high pressure homogenization, ball mill, Ultra Turrax are much more effective than ultra-sonication (Taucher et al, 2016).

Microscopic pictures of Chlorella sp., before and after using ultrasonic processor for 20 min are given in Fig. 3.

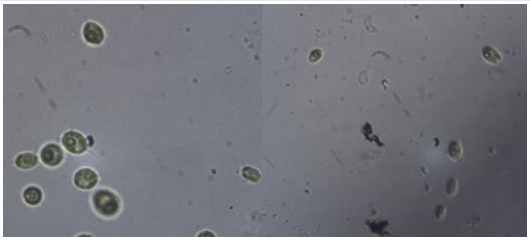


Fig. 3.Microalgae Chlorella Sp. before investigations and after investigations using high frequency vibrations

In order to achieve the highest efficiency of algae cells breaking using ultrasonic frequency in algae suspension, the enclosed system was used for toward research. Applying continuous ultra-sonication, the results of investigations showed that the highest efficiency of algae cell breaking was achieved after 20 min of operation (Fig. 4). As can be seen from the compared results with an open system, the highest efficiency of algae cell breaking was achieved using an open system (more than 3 times more efficiently). In order to achieve the maximum of broken algae cells and to use this approach in the industry, it is necessary to do some changes in this equipment.

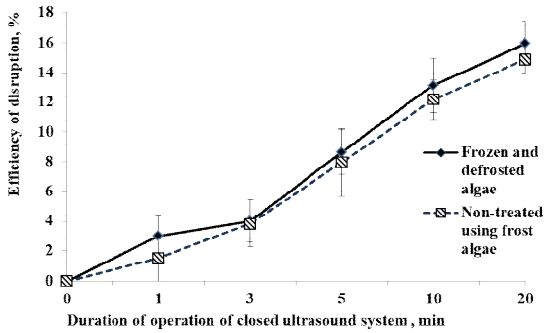


Fig. 4. Change in efficiency of algae disruption using a closed high frequency system

The research of algae cell breaking via an ultra-homogenizer were carried out using different rotor speeds of 16000, 20000 and 24000 rpm. The highest efficiency (reached nearly 35 %)was achieved treating algae suspension for20 min at 24000 rpm (Fig. 5).

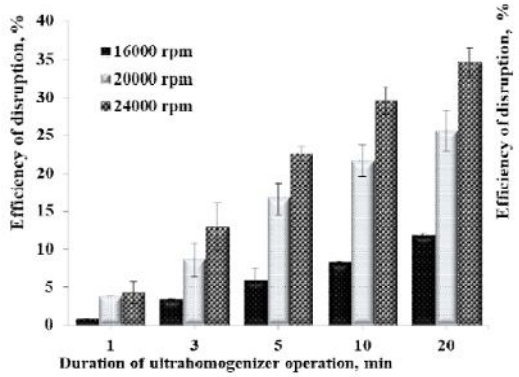


Fig. 5. Dynamics of algae cell disruption efficiency by usingultrasonic processor

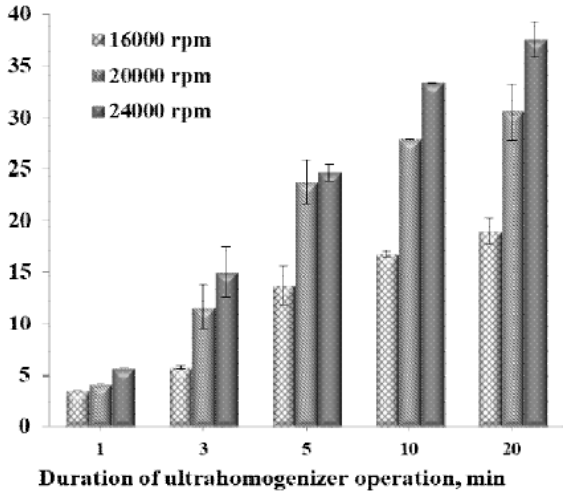


Fig. 6. Dynamics of frost-treated algae cell disruption efficiency by using ultra-homogenizer

The first 10 min of investigation showed that operating suspension with ultra-homogenizer from 1 to 10 min, the efficiency of algae cell breaking increases significantly. By prolonging duration of operation, rapidly growth of efficiency of algae cell breaking strongly decelerates (an increase from 4 to 5 %).Slightly higher positive breaking effect was achieved during research with treated using frostChlorella sp. (Fig. 6) (app. 38% for 20 min operation at 24000 rpm).

In presented study, the results of research demonstrate that ultra-sonication mode is more effective in breaking of microalgae Chlorella sp. cell compared to ultra-homogenization. Taucher et al (2016) published the opposite results with Haemotococcuspluvialis. Efficiency of breaking cell using Ultra Turrax (ultra-homogenizer) was reached app. 80 %, while using ultrasonication technique was only less than 10 %.

CONCLUSIONS

The efficiency of microalgae Chlorella sp. cell breaking using ultrasonication and ultra homogenization was investigated. Non-processed and treated using frost microalgae suspension was used in this study. It was estimated that suspension pre-treatment using frost enhances cell breaking efficiency from 1 to 9 %. The highest positive breaking effect (50.76 %) was achieved using the longest operation duration of suspension, i.e., 20 min. Comparing the results of investigations using both techniques was observed that app. 30 % higher disruptive effect is obtained with VCX 130 ultrasonic processor than using ultra homogenizer (rotor speed – 24 000 rpm). High efficiency of cell breaking is hard to reach because of the fact that cell walls of Chlorella sp. are very rigid and have three layers (Dodge, 1973).

ACKNOWLEDGEMENTS

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