INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 19, No. 5, p. 60-66, 2021

RESEARCH PAPER OPEN ACCESS

Comparison of different algal cell disruption methods

Fakhra Aslam*, Arifa Tahir, Iram Naz, Maryam Waseem

Environmental Sciences Department, Lahore College of Woman University, Lahore, Pakistan

Key words: Cell disruption, Microalgae, Ultrasonication (US), Freezing-thaw, Osmotic shock.

http://dx.doi.org/10.12692/ijb/19.5.60-66 Article published on November 30, 2021

Abstract

A wide range of different commercial products ranging from biofuels, biomolecules to nutraceuticals is associated with microalgae. The vital step is the disruption of the cell wall which assists in the release of intracellular products that are essential for the production of these products. The cell wall disruption process needs lots of energy and time. Various methods for rupturing the cell wall including mechanical and non-mechanical methods, have been used. Herein, a detailed review of possible cell disruption procedures of microalgae cells is provided, considering their benefits and drawbacks. This study investigated the use of ultrasonication, osmotic shock and freezing-thaw method as laboratory-scale disruption methods for microalgal cells. The cell disruption degree was investigated and the cell morphology before and after disruption was assessed with scanning and transmission electron microscopy. UV absorbance (260 nm) was used as the quantification method to compare the cell wall disruption rate. The highest disruption degree, up to 100 %, was achieved by the freezing-thaw method to achieve intra-cellular proteins.

* **Corresponding Author:** Fakhra Aslam ✉ fakhra_chaudry@yahoo.com

Introduction

Algae are photosynthetic organisms that can live in both fresh and saline environments ranging from unicellular (phytoplankton or microalgae) to multicellular (filamentous or macroalgae) (Bharathiraja *et al.*, 2015; Sambusiti *et al.*, 2015). Microalgae is a source of many useful nutrients. The bioaccessibility of these nutrients, such as lipids, proteins, carbohydrates and vitamins, depends upon its structurally complex cell wall (Canelli *et al.,* 2021). The disruption and disordering of algae cells are essential for the extraction of intracellular components and retrieval of targeted products, especially biological products (Phong *et al.*, 2018). Moreover, the quality of desired components is subjected to applied cell disruption methods; suitable methods should be carefully selected depending on the utilization purpose (Spiden *et al.*, 2013). Specific and precise cell disruption techniques are needed for the efficient extraction of lipids; some of the employed methods include bead beating, mechanical pressing, homogenization, microwave, sonication, pulsed electric field, and osmotic shock (Cooney *et al.,* 2009).

Disruption is a primary and essential step in any research which includes isolating, analyzing or separating some constituents from an intact sample. Physical and mechanical methods which rely on grinding, beating, shocking, and shearing can be employed for chemically resistant samples (Burden, 2012). The aim of this study is to investigate the cell disruption effectiveness of *Scenedesmus sp*. using three disruption methods, Ultrasonication (US), osmatic shock, and freeze-thawing, to estimate the cell disruption with UV absorbance (260 nm).

Material and methods

Cell wall disruption by ultrasonication

Pre-treatment of algal strains was carried in order to make dilutions for ultra-sonication. The thick algal biomass was diluted with normal saline media (0.9 %) as one loop of biomass per 1 ml to make dilution up to 25 ml in a centrifuge tube (Safi *et al.*, 2014. The ultra-sonication probe was immersed into the center of the diluted *Scenedesmus* suspension and ultrasonication was carried at 40 Hz. Experiments were conducted in batch mode with varying treatment times (Halim *et al.,* 2012).

Cell wall disruption by osmotic shock

An aliquot (5ml) of the thick cell biomass of a *Scenedesmus sp*. was blended with 50ml of distilled water for the osmotic shock using 10 % NaCl solution with a vortex for 1 min and was maintained for 48 hours (Lee *et al.,* 2010).

Cell wall disruption by the freezing-thaw method

1ml of a *Scenedesmus* biomass was dissolved in 10 mL of distilled water, frozen at −20 °C for 90 min and thawed, 3 freeze-thaw cycles in total were performed (Zhang *et al.,* 2018).

Quantitative evaluation of cell disruption

UV absorbance of Scenedesmus suspension supernatant was measured by a UV-Vis spectrophotometer (Model: UV-2800 Hitachi) at 260 nm using a 1 cm path length quartz cell. The supernatant was obtained by centrifuging the microalgae suspension at 14,000 rpm for 30 min. The untreated and treated microalgae suspensions (Chlorella & Scenendesmus sp.) were freeze-dried and subjected to observation using an SEM.

Data analysis

The data on cell disruption were analyzed according to a normalization method (Spiden *et al.,* 2013). The purpose of the normalization is to facilitate the comparison of the utility of the indicator. For UV absorbance (260 nm), which represented the released cell metabolite quantity, the cell disruption rates were calculated using the following equation

$$Dt = xt - xi / xmax - xi * 100$$

Where xt represents the metabolite concentration at point t, xi represents the initial metabolite concentration in the suspension supernatant, and xmax represents the maximal observed cell metabolite release.

Results and discussion

In an ultrasonic treatment, a cavitation process is initiated as a result of energetic acoustic waves of higher frequency and transmitting a shock wave in the immediate medium causing cell disruption through high shear forces (Mendes *et al.,* 2001). The results of ultrasonic cell disruption of *Scenedesmus sp*. using UV absorbance at different time periods are presented in Fig. 1. As depicted in Fig. 1, the disruption rate increased as the processing time increased with a distinct tendency: the highest *Scenedesmus sp*. cell disruption proportion of 99.3% was obtained respectively at the processing time of 25 min and the ultrasonic power of 220W.

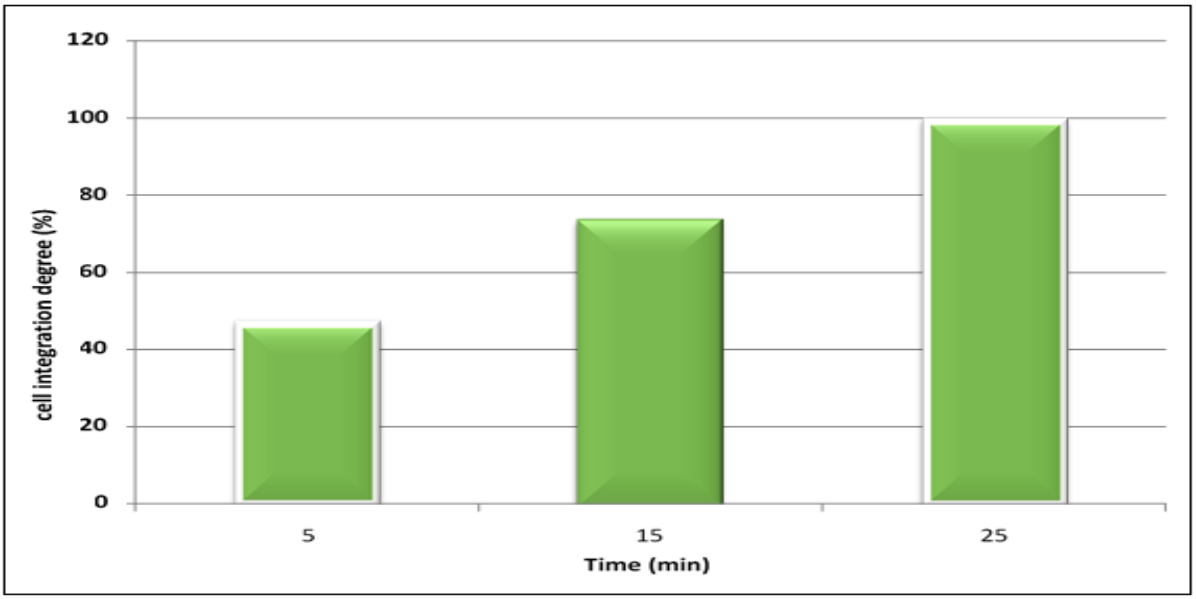

Fig. 1. Effect of ultrasonication treatment on the disruption degree for *Scenedesmus sp*. at 40 kHz.

The study revealed that ultrasonic power of (220 W) was a threshold for maximizing the permeability of *Scenedesmus sp*. cells by moderately disrupting the surface barriers without completely disintegrating it. The UV absorbance (260nm) was used as the quantification method; the higher absorbance value indicates the greater disruption of the cell wall. The SEM result of controlled and ultrasonicated *Scenedesmus sp*. for 25 min is shown in Fig. 2.

The extreme ultrasonic energy exposure and mechanical energy of cavitation torn the cells into minor fragments. The transformation of cell shape from round to uneven and solid mass reduction led to reduced turbidity. The release of cellular metabolites like protein, nucleic acid, chlorophyll and lipid from cell breakage in the culture medium is responsible for the increased UV absorbance. Free radicals formed from the reactions of water and ultrasonic waves subsequently subjected the metabolites to oxidation (Wang *et al*., 2014).

The abrupt reduction in the concentration or movement of water through the algal cell membrane is known as osmotic shock.

The *Scenedesmus* biomass was treated with 5 and 10 % NaCl solution to observe the disintegration rate of cell wall disruption. The microscopic results of osmotic shock and control are shown in Fig. 3. Using UV-VIS spectrophotometer, a 95 % disintegration rate was observed with 10% NaCl after 48 hr through osmotic shock.

In order to retain the movement of water across the algal cell wall, it was run at the vortex for 1 min and maintained for 48 hr. The *Scenedesmus sp*. gave an appropriate efficiency rate of cell disruption (95%) with 10% NaCl to release intracellular products including proteins, lipid, carbohydrates and others. The osmotic shock method is simple, but it is a time-consuming method (48 hr) for treatment and showed similar results to the bead-beating method (Lee *et al.,*

2010). The stress from the fast change in movement produced by adding high applications of a solute or other additive (e.g., substrates salt, neutral polymers, such as dextran, polyethylene glycol) results in rupturing the cells, releasing the cellular and intracellular components (Mercer and Armenta 2011).

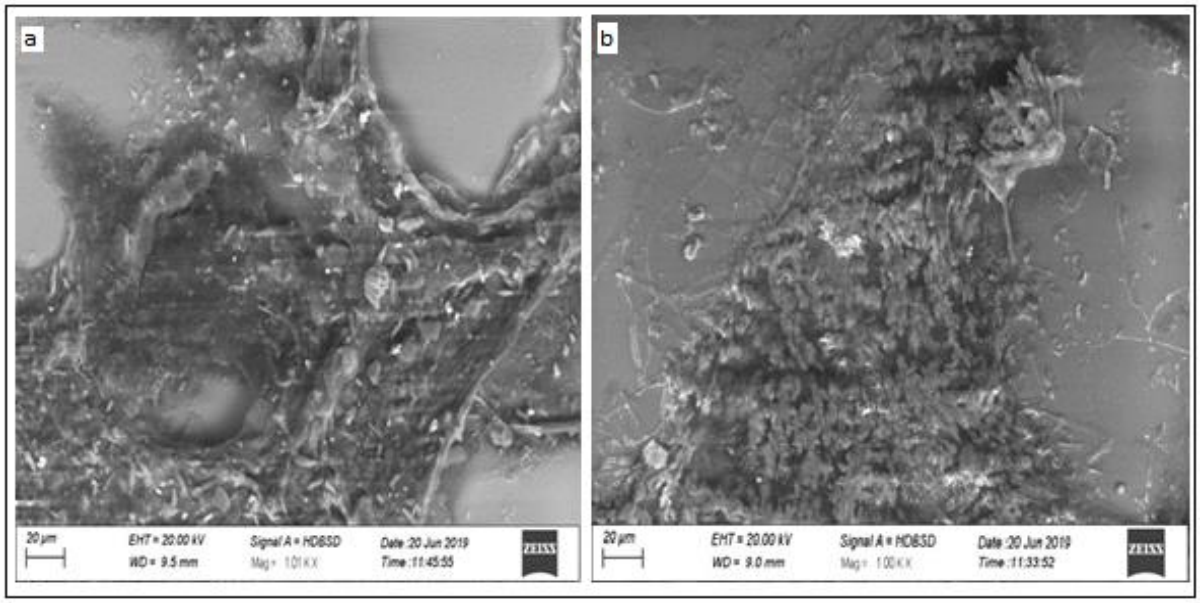

Fig. 2. Scanning electron microscope (SEM) micrographs of *Scendesmus sp* before and after treatment: (a) before treatment; (b) US: 25 min, 260 W, 40 kHz.

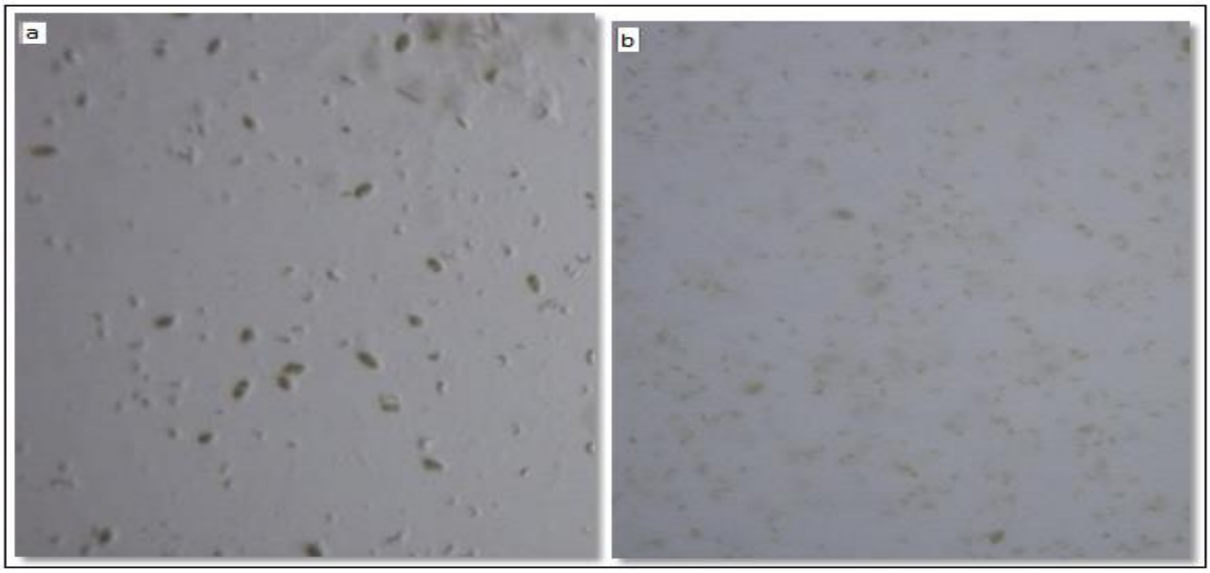

Fig. 3. Microscopic results of *Scendesmus sp* before and after treatment: (a) before treatment; (b) Osmatic Shock.

The freezing and thaw method was carried out at −20 °C for 90 min by using the Freeze-thaw cycle—the results displayed through the microscope slide at a light microscope. Fig. 4 shows the cell wall status of controlled and disrupted cells of *Scenedesmus* sp. The highest *Scenedesmus sp*. disruption proportion of 100 % was obtained with a processing time of 90 min at -20°c including 3 freeze-thaw cycles. Fig.e 4 shows the cell wall status of controlled and disrupted cells of *Scenedesmus* sp. The morphological changes witnessed by the microscope presented the existence of various irregular cells in the supernatants of solvent extraction, proposing the disruption of the cell wall of algae. The evaluation of disintegration

degree of 100 % by UV absorbance at 260nm, giving maximum absorbance value in UV spectrophotometer. The UV absorption spectra confirmed the presence of a large amount of proteins/peptides (absorption peaks at the wavelength 220–280 nm), and some pigments (lutein or chlorophyll, absorption peaks at wavelengths 410 nm or 640 nm) (Zhang et *al*., 2018).

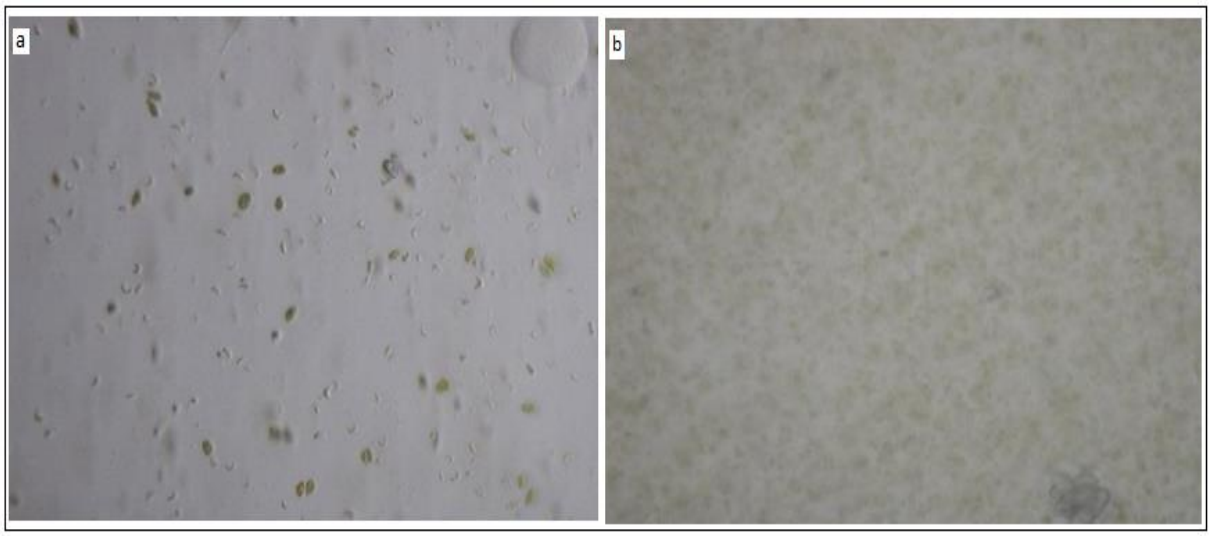

Fig. 4. Microscopic results of *Scendesmus sp* before and after treatment: (a) before treatment; (b) freeze and thawing.

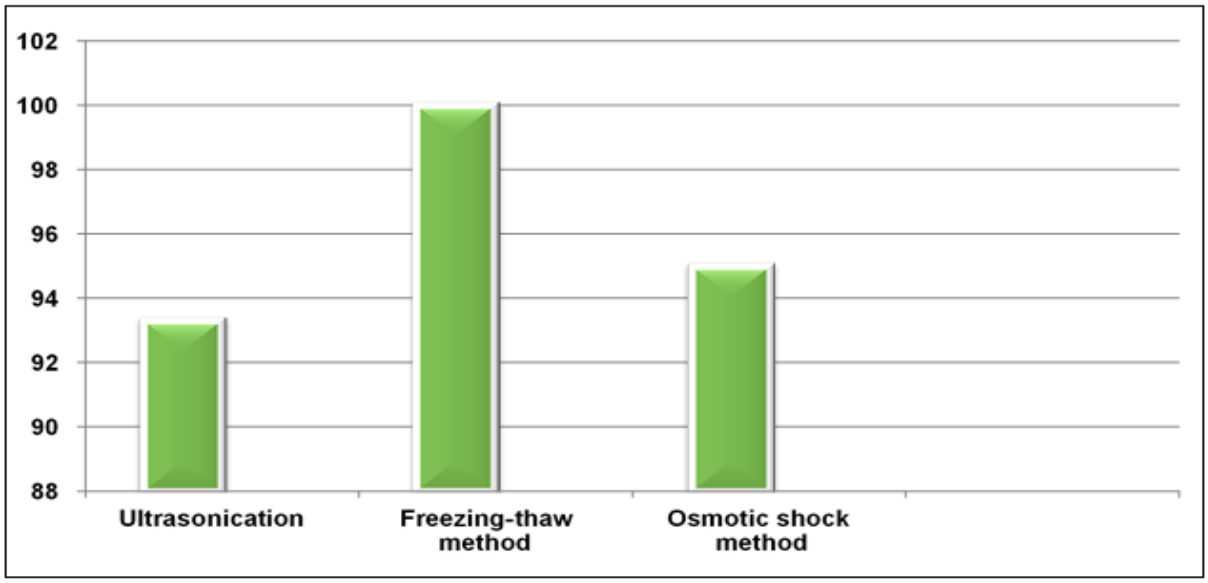

Fig. 5. Comparison of different physical methods for cell wall disruption of *Scenedesmus sp*.

The comparison of percentage cell disintegration by different physical methods (ultrasonication, osmotic shock, freezing-thaw method) for *Scenedesmus* sp. is shown in Fig. 5. The major drawback of microalgae biomass ultrasonication is that the efficiency of cell disruption for some species is comparatively low. Temperature is one of the variables that control the quality of the product. However, it reduces the effectiveness of cell disruption. The probability of combining ultrasonication with other solvent systems to enhance the efficiency and reduce the energy need remains interesting in the case of mild microalgae (Sheng *et al.,* 2012).

The production cost and energy consumption can be reduced by implementing the osmotic shock method.

The other benefit of the osmatic shock method is the recycling of resultant wastewater by reverse osmosis technology. The implementation ease, mild nature of the method and the potential to release products are the main attractions of the freeze and thaw method (Arnal *et al.*, 2005). Microalgae are gifted vehicles for biodiesel production and own advantages like higher productivity and growth rate and tendency to grow in different environments (fresh, brackish, or saltwater). In comparison to other conventional crops, microalgae oil productivity (20%–50% by dry weight basis) is higher (Singh *et al.,* 2011). The selection of effective microalgae species along with suitable cell wall disruption methods for lipid extraction is essential for commercial biodiesel production (McMillan *et al.,* 2013, Griffiths, M.J *et al.*, 2009).

Conclusion

This study investigated the effectiveness and efficiency of the various physical method of cell disruption for *Scenedesmus sp*. freezing-thaw method is the most effective physical method with higher efficacy as compared to the US. UV absorbance was used as an indirect quantitative method, but it is not appropriate in the case of significant metabolite degradation. In order to maximize the disruption and avoid any detrimental effects on preferred products, careful control of treatment conditions is needed while applying physical methods.

References

Arnal JM, Sancho M, Iborra I, Gozálvez JM, Santafé A, Lora J. 2005**.** Concentration of brines from RO desalination plants by natural evaporation. Desalination **182,** 435–439.

Bharathiraja B, Chakravarthy M, Kumar RR, Yogendran D, Yuvaraj D, Jayamuthunagai J. 2015. Aquatic biomass (algae) as a future feed stock for bio-refineries: a review on cultivation, processing and products. Renewable Sustainable Energy Reviews **47,** 634–653.

Burden DW. 2012. Guide to the disruption of biological samples. Random Primers **12(1),** 1-25.

Canelli G, Murciano Martínez P, Maude Hauser B, Kuster I, Rohfritsch Z, Dionisi F, Mathys A. 2021. Tailored enzymatic treatment of Chlorella vulgaris cell wall leads to effective disruption while preserving oxidative stability. LWT-Food science and technology 143.

Cooney M, Young G, Nagle N. 2009. Extraction of bio oils from microalgae. Separation & Purification Reviews **38(4),** 291-325.

Cooney M, Young G, Nagle N. 2009. Extraction of bio–oils from microalgae. Separation and Purification Reviews **38,** 291–325.

Griffiths MJ, Harrison ST. 2009. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. Journal of Applied Phycology **21(5),** 493-507.

Halim R, Harun R, Danquah MK, Webley PA. 2012. Microalgal cell disruption for biofuel development. Applied Energy **91(1),** 116-121.

Henderson R, Parsons SA, Jefferson B. 2008.The impact of algal properties and pre-oxidation on solid–liquid separation of algae. Water research **42(8-9),** 1827-1845.

Lee JY, Yoo C, Jun SY, Ahn CY, Oh HM. 2010. Comparison of several methods for effective lipid extraction from microalgae. Bioresource technology **101(1),** S75-S77.

McMillan JR, Watson IA, Ali M, Jaafar W. 2013. Evaluation and comparison of algal cell disruption methods: microwave, waterbath, blender, ultrasonic and laser treatment. Applied energy **103,** 128-134.

Mendes-Pinto MM, Raposo MFJ, Bowen J, Young AJ, Morais R. 2001. Evaluation of different cell disruption processes on encysted cells of Haematococcus pluvialis: effects on astaxanthin recovery and implications for bio-availability. Journal

of Applied Phycology **13(1),** 19-24.

Mercer P, Armenta RE. 2011. Developments in oil extraction from microalgae. European Journal of Lipid Science and Technology **113(5),** 539-547.

Phong WN, Show PL, Ling TC, Juan JC, Ng EP, Chang JS. 2018. Mild cell disruption methods for bio-functional proteins recovery from microalgae—Recent developments and future perspectives. Algal research **31,** 506-516.

Safi C, Ursu AV, Laroche C, Zebib B, Merah O, Pontalier PY, Vaca-Garcia C. 2014. Aqueous extraction of proteins from microalgae: effect of different cell disruption methods. Algal Research **3,** 61-65.

Sambusiti C, Bellucci M, Zabaniotou A, Beneduce L, Monlau F. 2015. Algae as promising feed stocks for fermentative biohydrogen production according to a biorefinery approach: a comprehensive review. Renewable and Sustainable Energy Reviews **44,** 20-36.

Sheng J, Vannela R, Rittmann BE. 2012. Disruption of Synechocystis PCC 6803 for lipid extraction. Water Science and Technology **65(3),** 567-573.

Singh A, Nigam PS, Murphy JD. 2011. Renewable fuels from algae: an answer to debatable land based fuels. Bioresource technology **102(1),** 10-16.

Spiden EM, Scales PJ, Kentish SE, Martin GJ. 2013. Critical analysis of quantitative indicators of cell disruption applied to Saccharomyces cerevisiae processed with an industrial high pressure homogenizer. Biochemical engineering journal **70,** 120-126.

Spiden EM, Scales PJ, Kentish SE, Martin GJO. 2013. Critical analysis of quantitative indicators of cell disruption applied to Saccharomyces cerevisiae processed with an industrial high pressure homogenizer. Biochemical Enginering journal **70,** 120–126.

Wang M, Yuan W, Jiang X, Jing Y, Wang Z. 2014. Disruption of microalgal cells using high-frequency focused ultrasound. Bioresource technology **153,** 315-321.

Zhang R, Chen J, Zhang X. 2018. Extraction of intracellular protein from Chlorella pyrenoidosa using a combination of ethanol soaking, enzyme digest, ultrasonication and homogenization techniques. Bioresource technology **247,** 267-272.