Low power ultrasound treatment for the enhanced production of microalgae biomass and lipid content

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ABSTRACT

The present study demonstrated the effect of ultrasound treatment on biomass, lipid and total lipid production of *Scenedesmus* sp. The microalga used in this study was treated with different ultrasonic power (0–50 W), ultrasonic time interval (0–5 s) and time (0–10 min). The significant improvement on biomass and lipid content was achieved at 20 W ultrasonic power, 2 s ultrasonic time interval and 4 min ultrasound. The overall lipid production was increased from 0.76 to 1.31 g/L under optimized ultrasonic conditions. On other hand, the Chl-*a*, carotenoid contents and oxygen evolution rate were also increased after the ultrasonic treatment with subsequent improvement in the efficiency of light capturing capacity, defense mechanism and oxygen evolution rate of cells leading to enhanced biomass and lipid production. The optimized ultrasound treatment enabled *Scenedesmus* sp. to increase its biomass, lipid and total lipid production. Hence proper ultrasound treatment on microalgae is an effective method improve the biomass and lipid content considerably.

1. Introduction

Due to an increasing demand of fuel needs, it is necessary to implement microalgae-based biodiesel as an alternative for traditional fossil fuels (Sivaramakrishnan et al., 2019; Mathimani and Pugazhendhi, 2019). Microalgae have several advantages such as ease of cultivation, embedding high levels of lipid and less requirement of land (Sivaramakrishnan and Incharoensakdi, 2018a; Kumar et al., 2019). To improve the lipid production and cell biomass in microalgae various strategies have been used such as culture medium components optimization like trace elements, carbon source and salts, physical parameters such as temperature, pH and light intensities, and culture mode type such as phototrophic, heterotrophic and mixotrophic growth (Lan Chi et al., 2019). Microalgae can be cultivated by using photobioreactor or in open ponds by utilizing carbon dioxide and light as an energy source, which is an added advantage on sequestrating the carbon molecules from atmosphere (Yan et al., 2016). Nutrient stress can improve the biomass and lipid content in microalgae. Nitrogen starvation improves the lipid content; however, it reduces the biomass content. Addition of sodium carbonate higher than the normal level in medium increases both the biomass and lipid content in microalgae (Sivaramakrishnan and Incharoensakdi, 2017a). Under nitrogen starvation, supplementation of sodium bicarbonate improves the lipid content in *Scenedesmus* sp. CCNM 1077 (Pancha et al., 2015). When comparing the cultivation of microalgae under heterotrophic, mixotrophic and photoautotrophic mode, the mixotrophic mode is superior to the other two modes in terms of cell biomass and lipid content (Sharma et al., 2018, 2019). In addition, ultrasound is a physical mode which has been applied in the microalgae industry nowadays. Several studies also report that the microalgae with ultrasound treatment have increased biomass and lipid contents as well as increased lipid extraction yield (Sivaramakrishnan and Incharoensakdi, 2017b; Sivaramakrishnan and Incharoensakdi 2019; Rajasekhar et al., 2012). Unlike physical treatment, the treatment with hydrogen peroxide (Sivaramakrishnan and Incharoensakdi, 2017c) and phenolic compound (Sivaramakrishnan and Incharoensakdi, 2018b) also improves the biomass and lipid content.

Ultrasound was used to rupture the individual cell walls causing the release of valuable products from the cells (Xiao and Ju, 2018). Ultrasound irradiation improves the enzyme activity, cell permeability and substrate transportation (Sivaramakrishnan and Muthukumar, 2012). Ultrasound induced microalgae-based biodiesel production is widely studied (Sivaramakrishnan and Incharoensakdi, 2017d). Ultrasound can even be used in the extraction of lipid from the microalgae cells (Han et al., 2016a). The growth of *Anabaena variabilis* under phototrophic condition was stimulated using ultrasound treatment (Han et al., 2016b). However, very few studies are available with regard to the improvement of biomass and lipid content in microalgae using

ultrasound treatment. A few studies on effects of ultrasonic treatment on lipid improvement and biomass changes have been reported (Ma et al., 2014; Rajasekhar et al., 2012). The change in permeability of cell membrane can be achieved by ultrasound, which alters the cell activity and product synthesis (Joyce et al., 2014). A change in biomass content or other cellular components by ultrasound also influences the photosynthetic pigments like Chl *a* and carotenoids. An increase in Chl *a* content increases the light capturing capacity of cells which enhances the photosynthetic rate and cell division, whereas an increase in carotenoid content can confer the good defense mechanism against the stress conditions. (Sivaramakrishnan and Incharoensakdi, 2018b).

The aim of this study is to use ultrasound treatment to improve the biomass and lipid contents in the isolated microalga *Scenedesmus* sp. The critical parameters for ultrasound treatment such as ultrasonic power, time interval and time duration on biomass and lipid contents were optimized. The lipid compositions and fuel properties were analyzed by adopting ASTM (American Society for Testing and Materials) methods.

2. Materials and methods

2.1. Microalgae and culture conditions

Scenedesmus sp. was obtained from our previous study (Sivaramakrishnan and Incharoensakdi, 2017a). The accession number of obtained *Scenedesmus* sp. was KR025877 (GenBank) which was isolated from the stone quarry pond water. The organism was maintained in the BG11 medium with continuous illumination of 50 μmol photons/m^2/s and continuous shaking at 100 rpm at 27 $\pm$ 1 °C. The purity of the culture was monitored regularly by observation under a microscope (Seek light microscope, Melbourne, Australia). The lipid, carbohydrate and protein content of the isolated *Scenedesmus* sp. was found to be 40, 22 and 19% respectively.

2.2. Experimental design

The effects of different ultrasonic power, time interval and time were explored to determine the optimal conditions for the positive effect on biomass and lipid content. The 12 days grown microalga was used for the ultrasonic treatment. The algal culture was treated by using Bandelin sonoplus 2200 device (Bandelin, Berlin, Germany). The treated cells were further harvested and washed thrice using autoclaved ultrapure water. The washed cells were further inoculated into fresh BG11 medium with initial cell density of OD_{730} = 0.05 and used for further investigations. The different ultrasonic power such as 0, 10, 20, 30, 40 and 50 W, different ultrasonic time interval 0, 1, 2, 3, 4 and 5 (s) and different ultrasonic time 0, 2, 4, 6, 8 and 10 (min) were used to determine the optimum conditions for the ultrasound treatment.

2.3. Biomass and lipid determination

The cell biomass was analyzed by determining dry cell weight (DCW). The determination of dry cell weight was done according to Sivaramakrishnan and Incharoensakdi, 2017a. The dry biomass was used for lipid content analysis using hexane as extraction solvent (Sivaramakrishnan and Incharoensakdi, 2017a). The lipid yield was calculated using the equation below.

Oil yield % = weight of oil extract (g)/weight of algal biomass (g) * 100.

2.4. Determination of photosynthetic pigments

The chlorophyll-*a* and carotenoid contents were determined according to the methods described by Moran (1982) and Pecker et al. (1992) respectively. The oxygen evolution rate was determined using Clark-type O_2-electrode (Hansatech instruments, UK) with a magnetic
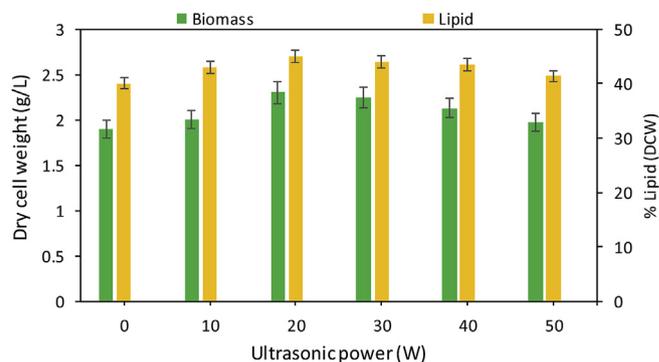

Fig. 1. Effect of different ultrasound power on biomass and lipid content. Data points indicating the mean of triplicate values and the error bars are standard deviations.

stirrer for homogeneous cell suspension (Baebprasert et al., 2011). The oxygen evolution rate is expressed as μmolO_2/mg Chl-*a*//h.

2.5. Statistical analysis

All the experiments were done in triplicates and the values are mean of three independent experiments. The shown error bars represent standard deviations (mean $\pm$ SD, n = 3). The graph pad software was used to analyze the statistical significance ($p < 0.05$).

3. Results and discussion

3.1. Effect of ultrasonic power on biomass and lipid content

The ultrasonic power was varied in the range of 0–50 W and the results were shown in Fig. 1. Increasing ultrasonic power from 0 to 20 W increased the dry cell weight considerably. A further increase in ultrasonic power, i.e. from 30 to 50 W slightly decreased the dry cell weight. Lipid content showed similar trend to that of biomass, both of which had maximum content at 20 W ultrasonic power. Previous studies reported that the 40–200 W ultrasonic irradiation induces the cell growth significantly in bacteria and microalgae (Han et al., 2016b; Wang et al., 2012). At high ultrasonic power, the cells cannot improve the biomass due to the inhibition caused by strong ultrasonic power. Joyce et al. (2014) reported the cell damage caused by the highly intensified power of ultrasound radiation. Besides, the ultrasonic cavitation caused by ultrasound could damage and deactivate the intracellular enzymes involved in the important metabolic processes linked with cell growth and lipid biosynthesis (Wang et al., 2012; Sivaramakrishnan and Incharoensakdi, 2018c). In the present study, the lipid content was increased under optimized ultrasound conditions. During ultrasound treatment, the harsh environment created in the bulk medium can cause temporary high temperature and pressure. Under such conditions, reactive oxygen species (ROS) may be generated and the ROS-derived electrons are utilized for lipid synthesis, thus resulting in high lipid content (Greenly and Tester, 2015). The maximum lipid yield of 1.03 g/L was achieved at 20 W power. Low power requirement is necessary to stimulate the cell growth which is an added advantage when compared to the previous studies.

3.2. Effect of ultrasound interval on biomass and lipid content

The different ultrasound intervals (0–5 s) were tested for their effects on the biomass and lipid contents in the *Scenedesmus* sp. It is necessary to find out the exact time interval for biomass and lipid content improvement. The maximum biomass and lipid content were obtained at 2 s, beyond which resulted in the decrease of the biomass and lipid production (Fig. 2). Suitable ultrasound irradiation causes the
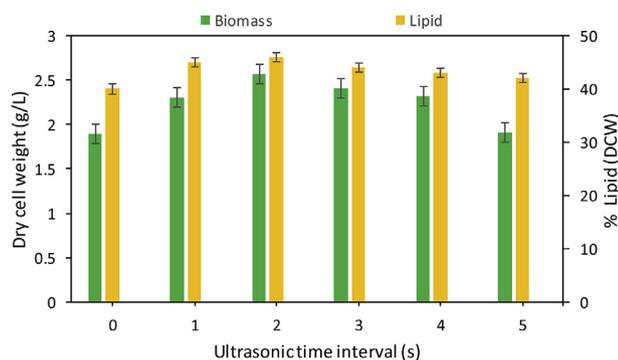
Fig. 2. Effect of different ultrasonic time interval on biomass and lipid content. Data points indicating the mean of triplicate values and the error bars are standard deviations.
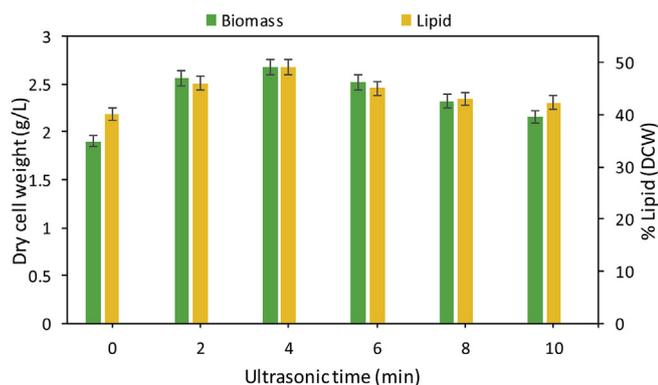

Fig. 3. Effect of different ultrasonic time on biomass and lipid content. Data points indicating the mean of triplicate values and the error bars are standard deviations.

cavitational effect and vibration on cell walls and separates the clumped cells resulting in improvement of the algal growth (Han et al., 2016b). However, prolonged ultrasound irradiation causes the damage on the intracellular enzymes involved in the lipogenesis, and thus affecting the lipid synthesis (Wang et al., 2012). In addition, prolonged ultrasound treatment increases the permeability of cells with the subsequent release of some important components necessary for the cell growth (Han et al., 2016b). Hence, proper ultrasound treatment is required to improve the cell growth without affecting the lipid content and permeability. It can be achieved with optimizing the time interval

and proper break between the ultrasound, which helps to improve the biomass and lipid contents without affecting the cell permeability. Prolonged irradiation causes serious damage to cells and hence it is important to set the time interval for ultrasound treatment.

3.3. Effect of ultrasound time on biomass and lipid content

Effects of different ultrasonic times on biomass and lipid contents were determined and shown in Fig. 3. The maximum biomass concentration and lipid content was obtained at 4 min ultrasound treatment time with 2 s time interval and 20 W power. The maximum of 2.68 g/L (DCW) biomass was obtained which is a 1.4-fold increase. The lipid content increased from 40% to 49% after the 4 min ultrasound treatment time. Increasing further ultrasonic treatment time tended to decrease both biomass and lipid contents. Appropriate ultrasonic treatment (4 min) improves the microalgae contact with light and nutrients which is important for the carbon and nitrogen fixation required for microalgae growth (Kumar et al., 2010). Further prolonging ultrasound to 10 min decreased the biomass and lipid contents to a lower level than that without treatment (Data not shown). Ultrasonic waves produce mild rupture on cell walls which improves the cell biomass by allowing the cells to contact the nutrients and light more effectively (Han et al., 2016a). Joyce et al. (2010) reported that the ultrasound treatment removes the clumps of microalgae which increases the cell biomass concentration. Hence, the positive effects of ultrasound treatment vary depending on the cell nature, ultrasound power and treatment time (Rajasekhar et al., 2012).

3.4. The total lipid production

The overall lipid production after ultrasonic treatment optimization was determined and shown in Fig. 4. The lipid production increased from 0.76 g/L to 1.31 g/L, a 1.7-fold increase in total lipid production. Increasing the ultrasonic power, time interval, and time above the optimum level decreased both biomass and lipid contents. To achieve the maximum lipid yield, it is necessary to select appropriate ultrasound conditions under which highest lipid and biomass contents can be obtained.

3.5. Effect of ultrasound treatment on photosynthetic pigments

The Chl-*a*, carotenoid and oxygen evolution rate were determined after the ultrasound optimization and shown in Fig. 5. Under the optimized condition, i.e. 20 W power, 2 s ultrasonic time interval and 4 min ultrasonic treatment, elevated Chl-*a* and carotenoid contents and
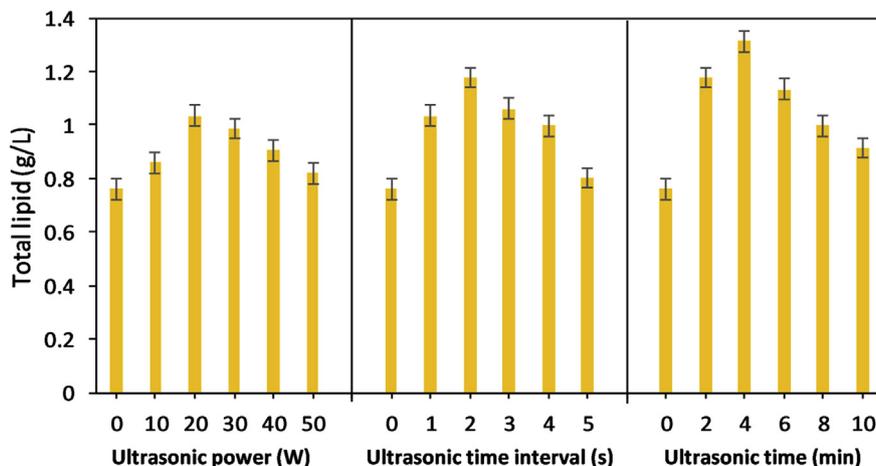

Fig. 4. Effect of different ultrasonic power, time interval and time on total lipid production. Data points indicating the mean of triplicate values and the error bars are standard deviations.
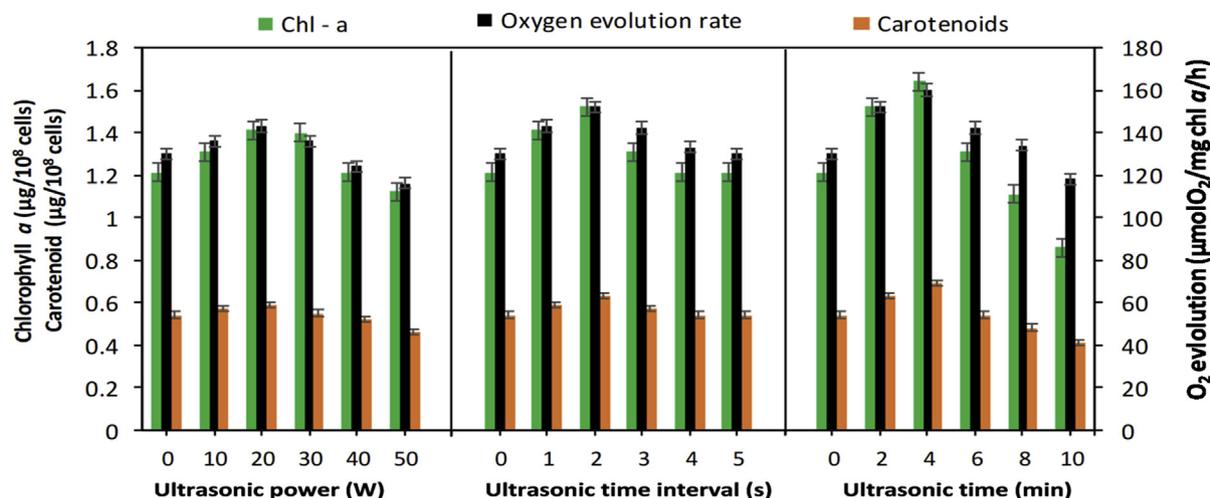
Fig. 5. Effect of different ultrasonic power, time interval and time on Chl-*a*, carotenoids and oxygen evolution rate. Data points indicating the mean of triplicate values and the error bars are standard deviations.

oxygen evolution rate were observed. Increasing ultrasonic treatment conditions decreased the photosynthetic pigments. During stress or physical treatment, the damage of chloroplast is likely, and thus Chl-*a* was reduced (Chokshi et al., 2017). Hence, increasing treatment conditions can affect the chloroplast of the cells and decrease the Chl-*a* content in the present study. Chl-*a* is the important component which improves the light capturing capacity of the cells which is important for the cell growth. Hence, appropriate ultrasonic treatment maintains the Chl-*a* content which is essential for cell growth.

Increasing ultrasonic power, time interval, and time did not show significant improvement in the carotenoid content (Fig. 5). The maximum carotenoid content was achieved at 4 min ultrasound time after the optimization of ultrasonic power and time interval. Altogether, the carotenoid content in most cases except under optimized conditions is close to the control. An increase in carotenoid displays a good defensive mechanism against stress or physical treatment (Paliwal et al., 2015). In the present study, the increase in carotenoid content under the optimized conditions indicates that the microalga used in this study can defend against the ultrasonic treatment.

The changes in oxygen evolution rate after the ultrasonic treatment were shown in Fig. 5. Increasing ultrasonic power, time interval, and time increased the oxygen evolution rate up to 20 W power, 2 s time interval and 4 min time respectively. Beyond the optimal conditions of ultrasound treatment, oxygen evolution rate tended to decrease. Oxygen evolution rate is an important indicator to determine the light harvesting capacity of the microalgae cells. Highest oxygen evolution rate results in highest photosynthetic efficiency (Ma et al., 2013).

The ultrasonic treatment with suitable conditions did not affect the microalgal cells used in this study, but showed positive consequences on biomass and lipid contents. Increasing light capturing capacity by ultrasonic treatment improves the biomass and lipid contents. Cells can defend against the stress environment by increasing carotenoid content. Hence, ultrasonic treatment is the potential method to improve the biomass, lipid content and total lipid production considerably.

4. Conclusions

The beneficial aspect of ultrasonic treatment was studied using *Scenedesmus* sp. The results clearly indicate that the 20 W ultrasonic power, 2 s time interval and 4 min treatment time showed maximum biomass (2.68 g/L), lipid (49%) and total lipid production (1.31 g/L). Increasing treatment conditions above the optimum level damages the cell capabilities and showed a decreasing trend of biomass and lipid contents. The Chl-*a*, carotenoid contents and oxygen evolution rate showed highest value under optimized conditions. The results demonstrated that the ultrasound treatment on cells could be the productive way to improve biomass and lipid contents which can be further utilized for biodiesel production.

Conflicts of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101230.

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