Bioresource Technology 152 (2014) 407–413

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

# An investigation of ultrasound effect on microalgal cell integrity and lipid extraction efficiency

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## HIGHLIGHTS

- Both studied algal cell integrity and solvent-based lipid extraction efficiency.
- Cell disruption was revealed by increased protein and carbohydrate concentrations.
- Sonication creates 1.5–2.0 fold increase in lipid extraction with solvents.
- Neutral lipid structure of the microalgae may change by ultrasound treatment.

## ARTICLE INFO

*Article history:*
Received 25 August 2013
Received in revised form 8 November 2013
Accepted 11 November 2013
Available online 19 November 2013

*Keywords:*
Ultrasound
Microalgae
Secondary effluent
Total lipids
Lipid extraction

## ABSTRACT

In this study, different ultrasound power intensities (0.1–0.5 W $mL^{-1}$) were applied at a frequency of 30 kHz and for durations of 5–60 min to mixed microalgal cultures, one cultivated in BG11 medium, and the other in secondary effluent wastewater. The ultrasonic effect on cell disruption was revealed by increased concentrations of protein and carbohydrate released into the solution, and a decreased concentration of total suspended solids in cell suspension. The highest intercellular material release was achieved at an ultrasonic energy intensity of 0.4 kWh $L^{-1}$, while the effect of ultrasound on cell disruption was reduced at higher energy intensities. Additionally, the ultrasonic effect on lipid extraction efficiency was studied in the presence of two different solvents, *n*-hexane and chloroform/methanol mixture. The application of ultrasound at 0.4 kWh $L^{-1}$, provided 1.5–2.0-fold increase in lipid extraction yields in the presence of the solvents.



## 1. Introduction

Microalgae, which are considered as eutrophication agents in natural water bodies, are now being evaluated as a new source of biofuel feedstock. The biomass and lipid productivities of these microorganisms are known to be much higher than those of terrestrial plants; yet no commercial production of algal biofuels has been realized at large-scale. In addition to massive water evaporation and possible microorganism contamination, earlier research indicates that meeting nutrient requirements of microalgal growth, and harvesting these microalgae and various algal constituents from water medium appear to be the most challenging tasks for large-scale production of these microorganisms (Christenson and Sims, 2011).

Several physical separation processes, including flocculation, floatation, filtration and centrifugation have been investigated for harvesting and dewatering of algal suspensions. Typically, algal biomass concentrated by using one or more of these processes is then subjected to extraction of algal cell contents, such as lipids and carbohydrates. These harvesting and dewatering steps could be economically undesirable as they require large capital and energy inputs (Uduman et al., 2010). Thus, there is a growing interest for direct extraction of algal metabolites that can by-pass prior harvesting and dewatering steps.

Extraction of cell contents directly from wet microalgal biomass thus emerges as one of the key parameters in producing sustainable biofuel from microalgae production (Adam et al., 2012). Several extraction methods have already been utilized for microalgal lipid extraction, some of which are based on extraction with solvents, including *n*-hexane (Halim et al., 2011), chloroform/methanol mixture (Bligh and Dyer, 1959), and methanol (Patil et al., 2011). Additionally, several solvent-free methods have been investigated, including the use of bead mills (Richmond, 2004; Lee et al., 2010), enzymes (Sander and Murthy, 2009), ultrasound (Lee et al., 2010; Adam et al., 2012; Sostaric et al., 2012), microwaves (Lee et al., 2010; Sostaric et al., 2012), autoclave, osmotic pressure (Lee et al., 2010), and supercritical $CO_2$ extraction (Halim et al., 2011; Sostaric et al., 2012).

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http://dx.doi.org/10.1016/j.biortech.2013.11.018

However, the so-called "solvent-free" methods are usually used in combination with a solvent to increase the extraction efficiency. In particular, sonication was observed to allow greater penetration of solvent into targeted biomass by producing acoustic cavitations in the solvent by the passage of ultrasonic waves (Kong et al., 2012).

Ultrasound-mediated extraction of lipids from algal biomass was tried earlier on dried biomass by using diethyl ether (Mecozzi et al., 2002), ethanol (Wiyarno et al., 2010; Wiyarno et al., 2011), *n*-hexane (Cravotto et al., 2008; Ranjan et al., 2010; Wiyarno et al., 2010; Wiyarno et al., 2011), chloroform/methanol mixtures (Lee et al., 2010; Ranjan et al., 2010; Araujo et al., 2011; Sostaric et al., 2012) and various other solvent mixtures (Araujo et al., 2013). Mecozzi et al. (2002) achieved higher lipid extraction efficiency from marine mucilage samples by sonication with diethyl ether compared to sonication with methanol. Wijarno et al. (2010) studied ultrasound-assisted extraction of algal lipids from *Nannochloropsis* sp., and found that the ultrasonic extraction was influenced by solvent types being used, and the use of ethanol required higher extraction temperature and longer duration compared to *n*-hexane. Cravatto et al. (2008), who used low frequency sonication for disruption of marine algal cells and *n*-hexane for solvent extraction, reported that the extraction yield of conventional method increased from 4.8% to 25.9% by application of ultrasound. Similarly, Ranjan et al. (2010) investigated the effect of ultrasound on Soxhlet (using *n*-hexane) and Bligh and Dryer (using a mixture of chloroform and methanol) at 20 kHz and 100 W sonication conditions. Sonication produced a 3-fold increase in the efficiency of Bligh and Dryer method; however, it did not affect the efficiency of the Soxhlet method significantly. So, it was claimed that the solvent selectivity was the most effective parameter on the extent of lipid extraction rather than the bulk convection created by ultrasound (Ranjan et al., 2010). Recently, Araujo et al. (2013) compared different solvent extraction methods with the assistance of ultrasound waves. The highest extraction of lipid from *C. vulgaris* was achieved when Bligh and Dyer method (chloroform/methanol) was applied in conjunction with 29.7 W $L^{-1}$ ultrasonic intensity.

These earlier studies on ultrasound-assisted lipid extraction with solvents seemed to have focused on the effect of solvent selection, rather than the effect of ultrasound application. The present study, on the other hand, has focused on the effect of ultrasound energy intensity on the efficiency of both cell disruption and extraction of cell contents in the presence of various solvents. Furthermore, primarily dried biomass was used in earlier studies, whereas wet biomass was used in the present study. In the first phase of the study, the change in cell integrity under different ultrasound energy intensities was investigated. Then under the most disruptive energy intensity, two well-known solvent extraction methods for lipids, namely, Bligh and Dryer (using chloroform/methanol) and Soxhlet Methods (using *n*-hexane), were applied to observe the effect of ultrasound on lipid extraction from wet microalgal biomass.

## 2. Methods

### 2.1. Culture media and microorganisms

The mixed microalgae culture used in these experiments was prepared by acclimation of algal strains collected from various water bodies to the secondary effluent a wastewater treatment plant (Ömerli Domestic Wastewater Treatment Plant, İstanbul, Turkey). This plant uses Sequencing Batch Reactors to treat 500 $m^3$ $day^{-1}$ of domestic wastewater biologically, without any additional advanced treatment for nitrogen (N) and phosphorus (P) removal. The effluent characteristics of the wastewater used as the algal growth medium are presented in Table 1 together with those of BG11 medium. The acclimation was carried out under approximately 150 μmol photon $m^{-2}$ $s^{-1}$ continuous illumination and at a temperature of 20 °C ± 2 in a 20 L glass container. After acclimation of 6 months, species from the *Chlorococcales* order of the *Chlorophyceae* class (i.e. *Scenedesmus* sp., *Chloroccum* sp.) were identified as the dominant group present in mixed culture cultivated in the wastewater medium.

### 2.2. Growth conditions

The algal cells acclimated to the secondary effluent were used as the inoculums for the enrichment of algae in two different media, for further experiments. The first medium was the same secondary effluent used for acclimation of mixed microalgae culture. The second medium was BG11, which is a widely used standard medium for algal growth. The BG11 medium was prepared according to Kim et al. (2011). The enrichment process was carried out in 20 L glass tanks under approximately 150 μmol photon $m^{-2}$ $s^{-1}$ continuous illumination and at 25 °C ± 2. In order to stabilize the pH between 6.0 and 7.0, and to supply adequate inorganic carbon for algal growth, pure $CO_2$ was injected through glass diffusers into the tanks. The $CO_2$ flow was adjusted by an automatic control system (WTW pH 296) to continuously maintain pH in the range of 6.0–7.0. The mixing in the tanks was provided by air diffusers located at the bottom of the tanks. When the biomass concentration reached 250 mg $L^{-1}$ or above in the enrichment media, biomass samples were taken from the tanks to be used for further experiments. The purpose of using two different media was to observe any effects of the growth medium in terms of cell disruption by sonication.

### 2.3. Cell disruption by sonication

Microalgae samples with a biomass concentration of 250 mg $L^{-1}$ were sonicated using an ultrasonic processor (Dr. Hielscher Gmbh, UP100H) equipped with a 8 cm-long and 7 mm-diameter tip sonotrode at a power and frequency of 50 W and 30 kHz, respectively. Of that sonotrode 5 cm was dipped into the working vessel from the center. Sonication was carried out for different sample volumes (100, 125, 166.7, 250 and 500 ml) and durations (5, 15, 30, 45 and 60 min). These sample volumes were selected to apply different ultrasound power intensities (0.1, 0.2, 0.3, 0.4 and 0.5 W $mL^{-1}$) on algal suspensions.

In order to identify the structural impact of the ultrasonic pretreatment at cellular scale, the cell morphologies of algal cultures were directly observed by a Scanning Electron Microscope (SEM) (FEI, Quanta 250 FEG) in high vacuum mode after coating the cells with gold. Before the SEM analysis, the algal samples were filtered

**Table 1**
The characteristics of secondary effluent and BG11 medium used in this study.

| Parameters | Secondary effluent | BG11 medium |
|---|---|---|
| pH | 7.42 | – |
| DO (mg $L^{-1}$) | 8.39 | – |
| Alkalinity (mg $L^{-1}$as $CaCO_3$) | 110 | – |
| COD (mg $L^{-1}$) | 34.1 | – |
| $BOD_5$ (mg $L^{-1}$) | 6.0 | – |
| $NH_3$–N (mg $L^{-1}$) | 0.24 | – |
| $NO_3^-$–N (mg $L^{-1}$) | 15 | 249.6 |
| $PO_4^{3-}$–P (mg $L^{-1}$) | 1.9 | 6.9 |
| TN (mg $L^{-1}$) | 17 | 250.0 |
| TC (mg $L^{-1}$) | 50.5 | 6.5 |
| IC (mg $L^{-1}$) | 37.8 | 2.3 |
| TOC (mg $L^{-1}$) | 12.7 | 4.2 |