Bioresource Technology 184 (2015) 291–296

Contents lists available at ScienceDirect

Bioresource Technology

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

Review

# Supercritical fluid extraction of valuable compounds from microalgal biomass

Hong-Wei Yen [a], Sheng-Chung Yang [b], Chi-Hui Chen [b], Jesisca [d], Jo-Shu Chang [c,d,e,*]

[a] Department of Chemical and Materials Engineering, Tunghai University, Taichung, Taiwan
[b] Metal Industries Research & Development Centre, Kaohsiung, Taiwan
[c] University Center for Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan
[d] Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan
[e] Research Center for Energy Technology and Strategy, National Cheng Kung University, Tainan, Taiwan

## HIGHLIGHTS

- SFE technology for recovering valuable compounds from microalgae is reviewed.
- Current status of lipids, PUFA, pigments recovery from algae with SFE is described.
- The strength and challenges of using SFE in microalgae biorefinery are discussed.

## ARTICLE INFO

*Article history:*
Received 31 August 2014
Accepted 7 October 2014
Available online 14 October 2014

*Keywords:*
Microalgae
Supercritical fluid extraction (SFE)
DHA
EPA
Pigments

## ABSTRACT

Many studies have demonstrated that the global demand for renewable biofuels, natural food pigments, and antioxidants has made microalgae a more attractive alternative resource. The application of supercritical fluid extraction (SFE) on the valuable compounds recovery from microalgal biomass has several advantages as compared to the conventional organic solvent extraction methods, especially for environmental considerations. This review presents comprehensive information on the current state of using SFE to recover valuable components from microalgal biomass, such as total lipids, long chain fatty acid and pigments, as well as the utilization and characteristics of the SFE technology. In addition, key factors and challenges that should be addressed during the application of SFE technology are also discussed. This report provides a useful guide that can aid in the future development of more efficient microalgae-based biorefinery process.



## 1. Introduction

The autotrophic cultivation potentials of microalgae for biofuels production, as well as for $CO_2$ emissions mitigation, have recently been realized. In addition to biofuels production derived from lipids, microalgae also have other valuable components, including long chain fatty acids (such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)) and pigments (such as astaxanthin and lutein), all which are worth further exploration. The global demand for alternative biofuels, as well as natural food pigments and antioxidants, have made their production via the cultivation of microalgae more attractive (Hu et al., 2013). Using microalgae with high lipid content for biodiesel production has been widely documented in the literature (Chen et al., 2011; Chen and Walker, 2011). The cultivation conditions for DHA and EPA production have also been demonstrated in the literature (Chi et al., 2007; Tang et al., 2011). Furthermore, the niche for producing natural pigments as antioxidants by using microalgae is becoming increasingly common. Some microalgae are currently used to produce pigments commercially; for example, *Haematococcus pluvialis* for astaxanthin (Thana et al., 2008), *Scenedesmus* sp. for lutein (Yen et al., 2011) and *Dunaliella salina* for β-carotene (Solana et al., 2014).

Recently, the concept of using microalgae as a renewable source for a variety of products has been gaining attraction (Demirbas, 2011; Norsker et al., 2011). Nevertheless, several problems need to be solved before the microalgae-based production technologies can become economically feasible. The major problems for the

* Corresponding author at: Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan. Tel.: +886 6 2757575x62651; fax: +886 6 2357146.

*E-mail addresses:* hwyen@thu.edu.tw (H.-W. Yen), yangsj@mail.mirdc.org.tw (S.-C. Yang), josephine@mail.mirdc.org.tw (C.-H. Chen), jesisca.cen@gmail.com (Jesisca), changjs@mail.ncku.edu.tw (J.-S. Chang).

http://dx.doi.org/10.1016/j.biortech.2014.10.030
0960-8524/

microalgae industry include high installing and operating costs, and the difficulty in controlling the mass culture conditions, especially for outdoor cultivation. Moreover, the most difficult problem to overcome is how to design efficient and low cost downstream processing, which generally accounts for the majority of manufacturing cost, to render the microalgae refinery process economically competitive (Yen et al., 2013). The extraction process of target compounds from microalgae is usually performed by using organic solvents, such as n-hexane; however, this chemical is toxic and has some pollution concerns. One alternative to traditional extraction by organic solvents is to carry out extractions using supercritical carbon dioxide (SC-$CO_2$), which is considered a safe, non-flammable solvent that offers a certain extent of tunable selectivity. The application of supercritical fluid extraction (SFE) to the recovery of valuable compounds is more attractive than others, especially from the consideration of environmental protection. Moreover, no follow-up separation step is needed after applying SFE since $CO_2$ is gaseous at ambient pressure, which allows for easy recovery of the extractant. Further, $CO_2$ can be recycled to avoid the concern of greenhouse effects. As a consequence, SC-$CO_2$ extraction of molecules of interest from microalgae is nowadays a subject of great interest and numerous experimental results have now been reported in the literature (Mouahid et al., 2013).

The operation of SFE is based on the solvating properties of supercritical fluid (SF), the characteristics of which are achieved by employing pressure and temperature that surpasses the critical point of the fluid. Indeed, SFE has immediate advantages over traditional extraction techniques, including a more flexibility by controlling SFE parameters, the avoidance of polluting solvents and a reduction of energy spent on post-extraction solvent recovery (Crampon et al., 2011). The extraction efficiency of applying SFE depends on some intrinsic tunable characteristics of the SF, such as temperature and pressure that both are important SFE operational parameters. In addition to these two intrinsic characteristics of SF, some extrinsic features including the characteristics of the sample matrix, interaction with targeted compounds and many environmental factors are also crucial to SFE extraction efficiency (Sharif et al., 2014). Due to the complex interactions between factors, a single SFE condition cannot generate enough information to appropriately address all affecting factors of the SFE process. To overcome this difficulty, a statistical experimental design involving response surface methodology (RSM) has often been adopted to help search for the optimum operation factors (Patil et al., 2011; Şanal et al., 2005). One of the most serious drawbacks of SFE is the higher equipment costs required compared to the traditional solvent extraction process. However, the basic process scheme (extraction plus separation) is relatively simple to scale up to industrial scale from the experimental scale, and thus has still gained interests (Reverchon and De Marco, 2006).

In this article, current information on applying SFE to the recovery of various compounds from microalgal biomass is reviewed. More specifically, detailed discussion on the application of SFE to recover total lipids, long chain fatty acids and pigments from microalgae biomass is provided.

## 2. SFE of total lipids and long chain fatty acids from microalgae

### 2.1. Total lipids

Due to the non-polar property of carbon dioxide molecules, SC-$CO_2$ is considered a suitable solvent for the extraction of lipids. Moreover, it has been reported that SC-$CO_2$ was selective for neutral lipids such as triglycerides, but did not solubilize phospholipids (Crampon et al., 2013; Mouahid et al., 2013). Therefore, the lipids obtained by SC-$CO_2$ extraction were mainly composed of triglycerides and a few of other compounds, such as free fatty acids, sterols, pigments and so on. Several SC-$CO_2$, extraction experiments have been reported, with extraction conditions and lipid yields reported in Table 1. It is known that the solubilization of SC-$CO_2$ can be modified by varying pressure (20–60 MPa), temperature (303.15–333.15 K), $CO_2$ flow rate (0.06–30 g/min), and extraction time (1–6 h). The extraction yield varies strongly with the operating conditions and can achieve nearly 100%. It has been reported that the extraction yield can increase significantly with an increase in pressure at constant temperature; however, the temperature factor is more complex because of the crossover phenomenon, which has been well-illustrated by several studies (Chatterjee and Bhattacharjee, 2014; Solana et al., 2014; Taher et al., 2014).

For microalgae samples, the water content is usually high. Thus, a drying step is usually required prior to extraction. However, the drying methods used may markedly influence the extraction kinetics. There are two well-known drying modes for microalgal biomass, namely, air-flow drying and lyophilization. As mentioned in the literature, lyophilization may preserve the microalgal cells' structure; however, the mass transfer would be limited by the diffusion of lipids through the cell membrane. Therefore, the mode of drying under air flow led to faster extraction kinetics (Crampon et al., 2013). However, lyophilization could play a role in decreasing cell rigidity while increasing the surface area and pore volume, which can enable more lipids to dissolve in SC-$CO_2$ (Chatterjee and Bhattacharjee, 2014; Taher et al., 2014). For SC-$CO_2$ extraction, it is suggested that the water content should be less than 20 wt% to achieve the best performance (Solana et al., 2014).

One of the important characteristics of microalgae is the rigidity of its cell wall, while damage to the cell wall allows more SC-$CO_2$ to reach the target components. There are several cell disruption methods, which can be divided into four categories: mechanical, physical, chemical and enzymatic approaches. In general, mechanical cell disruption methods are considered suitable processes due to the avoidance of product contamination and the ease for scaling up (Lee et al., 2012). Several process have been developed for cells rupture, such as bead milling, high-pressure disruption, microwave and ultrasonic (Chen et al., 2013; Cheng et al., 2011; Dejoye et al., 2011; Safi et al., 2014; Tang et al., 2011). Safi et al. (2014) indicated that bead milling pretreatment could increase the total extraction yield from *Chlorella vulgaris* by 16%, with similar results reported for high-pressure disruption pretreatment (Chen et al., 2013). Therefore, appropriate pretreatments are significant for the success of the extraction operation.

A major operational drawback in the use of SC-$CO_2$ is its low polarity, which greatly reduces the extraction efficiency of polar materials by using SC-$CO_2$ alone. Since polar lipids are hardly soluble in SC-$CO_2$, adding a small amount of modifiers is sometimes necessary. Although many polar solvents could serve as the modifiers, ethanol has generally been selected as the co-solvent due to its nontoxic property and good cell wall penetration to make neutral lipids more available to the non-polar solvent (Li et al., 2014), thereby increasing the extraction yield to up to 20–90% (Cardoso et al., 2012; Li et al., 2014; Nobre et al., 2013; Reyes et al., 2014).

### 2.2. Long chain fatty acids (DHA and EPA)

Polyunsaturated fatty acids (PUFA), particularly those with very long chains such as eicosapentaenoic acid (EPA; 22:5, n-3) and docosahexaenoic acid (DHA; 22:6, n-3), are widely recognized as important nutritional components that may help prevent various cardiac disorders. The ability of microalgae to accumulate EPA and DHA has been well known (Spolaore et al., 2006; Ward and Singh, 2005). The digestion of microalgae to accumulate DHA and EPA through the food chain has been the major route for presence