Algal Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Algal Research

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

Mild cell disruption methods for bio-functional proteins recovery from microalgae—Recent developments and future perspectives

Win Nee Phong[a], Pau Loke Show[b], Tau Chuan Ling[a], Joon Ching Juan[c,d], Eng-Poh Ng[e], Jo-Shu Chang[f,g,*]

[a] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Bioseparation Research Group, Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia
[c] Laboratory of Advanced Catalysis and Environmental Technology, Monash University Sunway Campus, Malaysia
[d] Nanotechnology & Catalysis Research Centre (NANOCAT), University of Malaya, 50603 Kuala Lumpur, Malaysia
[e] School of Chemical Sciences, Universiti Sains Malaysia, Minden 11800, Malaysia
[f] Department of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan
[g] Research Center for Energy Technology and Strategy, National Cheng Kung University, Tainan 701, Taiwan

ARTICLE INFO

Keywords:
Microalgae
Bio-functional proteins
Cell wall
Mild cell disruption

ABSTRACT

Bio-functional proteins from microalgae have numerous biological properties with health-promoting effects. However, efficient harnessing of bio-functional proteins from microalgae is still in its infancy. One of the major obstacles that hinder the mass production of bio-functional proteins is the presence of resistant cell wall that diminishes the liberation of cell contents. As the bio-functional proteins are very sensitive to denaturation, selecting a mild disruption method to rupture the cell wall, while preserving their bioactivity and functionality, is of vital importance in downstream processing. To ensure the future development of efficient mild disruption methods for maximum recovery of bio-functional proteins from microalgae, this review provides useful information on various mild disruption approaches, current status, potential technologies that are still under development, as well as their advantages and constraints. In particular, those potential technologies that require further attention in the future (namely, explosive decompression, microfluidization, pulsed arc technology and cationic polymer coated membranes) are also discussed in this review.

1. Introduction

A biorefinery is sustainable biomass processing, aiming to maximize the efficiency of resource utilization from the biomass feedstock. The co-production of a range of end products and energy from biomass in linear production chains can be achieved through the integration of biomass conversion processes and equipment [1–3]. The biorefinery approach enables the microalgal production to be economically feasible, by allowing the optimal harnessing of all the valuable compounds present in microalgae [3–6]. The implementation of the biorefinery concept in the microalgal industry is imperative in the creation of a sustainable and more environmental friendly future. The advantages include mitigating greenhouse gas emission, reduction in fossil fuel usage and overcoming the insufficiency of future food supply [2]. Microalgae are classified as the futuristic raw material in biorefinery process, because of their relatively untapped potential to produce multiple valuable products in addition to biofuels [2,7]. Although being small in size, these photosynthetic microorganisms have the capability of accumulating different types of metabolites, which can subsequently be transformed into value-added products [2]. The growing interest and increasing commercial demand in natural and healthy products in today's market trend has forced the development of novel products with valuable functional compounds from microalgae for food, nutraceutical and pharmaceutical industries [8]. Being a natural source of highly interesting biologically active compounds with positive health effects, microalgae produce a range of functional ingredients including polyunsaturated fatty acids, polysaccharides, natural pigments, essential minerals, vitamins, proteins, essential amino acids, and enzymes, as well as bioactive molecules [2,8,9]. Most of the functional components that possess health-promoting effects are associated with proteins, protein hydrolysates or peptides. Therefore, special attention has been paid to the bio-functional proteins and peptides from microalgae with a broad spectrum of biological properties such as antioxidants, antihypertensive, anticoagulative, antitumor

* Corresponding author at: Department of Chemical Engineering, Research Center for Energy Technology and Strategy, National Cheng Kung University, Tainan 701, Taiwan.
E-mail address: changjs@mail.ncku.edu.tw (J.-S. Chang).

http://dx.doi.org/10.1016/j.algal.2017.04.005
Received 20 May 2016; Received in revised form 4 April 2017; Accepted 10 April 2017

and immuno-stimulant activities [10].

The utilization of microalgae as source of high-value metabolites at commercial scale is still in its nascent form [11]. Extensive efforts have been dedicated to the development of cost-effective and feasible upstream processing particularly in the cultivation system [12], but still very limited reports on the recovery of high-value metabolites especially bio-functional proteins from microalgae are being observed. As the microalgal morphology is different from terrestrial plants, intracellular components cannot be extruded effectively from microalgae using a mechanical press, a method which is designed specifically for product extrusion from terrestrial crops, such as soy [13]. On top of that, there are many different species of microalgae grown under different cultivation conditions, varying greatly in their cell wall structure and chemical compounds concentration, making the predictions or extrapolations on disruption efficiency and the recovery of intracellular compounds impossible [1].

Biorefinery techniques, mainly focusing on downstream processes, are necessary to exploit all cell contents produced by microalgae after cultivation [3]. Downstream processing represents a major economic limitation to the mass production of high-value metabolites from microalgae at lower cost [1]. Downstream processing costs typically contribute to a big portion of the total cost, hence the development of competent and vigorous new downstream strategies is imperative to maximize product recovery from microalgae and favour the economic feasibility of the process [14,15]. One of the major problems faced in developing a suitable downstream strategy is the low recovery of high-value metabolites, limited by the rigid nature of microalgal cell wall. The structurally small microalgae are covered with multiple layers of resistant thick cellular walls that hinder the liberation of cell contents [1].

To overcome this chemical and structural barrier that limits the liberation of intracellular components, employing an appropriate cell disruption technique prior to extraction is undoubtedly one of the most crucial preliminary steps in downstream processing for the maximum recovery of cell contents from microalgae [1,16]. It is essential to ensure that the cell structure has been completely disintegrated to increase the efficiency of the extraction process [16]. However, selecting an ideal disruption method to facilitate the high-value metabolite extraction based on the biorefinery concept is quite challenging. The quantity and quality of functional compounds in the extract depends on the effectiveness of the cell disruption method [17,18]. The nature and composition of the structural cell wall has an important effect on the disruption efficiency and the extraction yield of intracellular biomolecules [18,19]. For example, lipid extraction from *Chlorella* or *Nannochloropsis* is much more difficult than *Dunaliella* due to the presence of a thick resistant cell wall [19]. Therefore, the choice of disruption technique is highly specific and strongly depends on the microalgal strain and the characteristics of the cell wall structure.

In addition to considering the structural morphology of each microalgal cell wall, the selection of a suitable cell disruption technique also largely depends upon the nature of the desired product or final product application [20]. Protein denaturation can be defined as the irreversible loss of the three dimensional structure due to the breakage of non-covalent bonds brought about by heat, alcohol, acids, bases, salts of heavy metals or other agents [21]. Most bio-functional proteins, such as enzymes with specific bioactivities, are very sensitive to denaturation; even slight changes in pH or temperature may cause inactivation and result in the loss of their biological function [21]. Therefore, achieving a good extraction yield of proteins from microalgae without degrading them is quite challenging [22]. If the target product involves the production of fragile functional compounds, the selection of a mild downstream processing without negatively affecting the activity and quality of the cell components of interest is required. Gentle breakage of the outer cell wall is necessary to promote the release of bio-functional proteins and peptides from microalgae, while preserving the functionality and biological activity of the molecules. As such, transforming biomass into a variety of high value-added products can be achieved in microalgal-based biorefinery [3].

Due to the growing significance of producing high-value compounds for food and pharmaceutical sectors based on biorefinery concept, the current research attention toward microalgae should predominantly focus on mild disruption methods. This is imperative to ensure the feasible and sustainable development in food and pharmaceutical industries. Despite the necessity, extensive and comprehensive studies on techniques to degrade cell walls of microalgae are still limited to date [1]. Hence, this review aims to provide useful information on various mild cell disruption methods, the current status, the potential innovative technologies that are still under development and require further attention in the future, as well as their benefits and constraints.

2. Microalgae

2.1. Types of microalgae

Microalgae can be grouped nutritionally on the basis of their energy sources, namely, autotrophic, heterotrophic or mixotrophic. Most microalgae are autotrophic, with the absolute requirement for light to perform photosynthesis, adequate supply of carbon dioxide and inorganic nutrients for optimal growth [23]. With the presence of these simple inorganic substances in their surroundings, autotrophs are capable of producing complex organic compounds, such as carbohydrates, fats and proteins [11]. On the other hand, some microalgae are heterotrophic. Heterotrophs are in contrast with autotrophs; they cannot fix carbon and therefore need organic carbon compounds, such as glucose, acetate, lactate and glutamate as carbon and energy source for growth. Microalgae that may have the dual capacity of both autotrophic and heterotrophic characteristics are known as mixotrophic. These phototrophic microalgae are able to adapt their metabolism to heterotrophic conditions, depending on the availability of organic compounds and light intensity [23].

2.2. Major chemical composition of microalgae

Microalgal biomass is composed of three main components: proteins, carbohydrates and lipids [15]. Studies on various microalgae demonstrated that protein is always the major constituent of the microalgae biomass (typically 25–40% of the dry weight) [23], followed by lipid and carbohydrate [24]. Microalgae have the ability to synthesise all types of amino acids, which are mostly equivalent or even better than that of other high-quality plant proteins [25]. Many metabolic studies have confirmed the capacity of microalgae as a novel source of protein in food [25] due to their abundance and complete amino acid profile.

2.3. Cell wall structure of microalgae

Microalgae are microscopic single cell microorganisms covered with a relatively recalcitrant cell wall and the intracellular compounds are mostly located in globules or bound to complex membranes, making the extraction of cell contents a great challenge [1,26]. The microalgal cell wall is a complex entity to preserve the integrity of the cell and serves as the main protective barrier against invaders and harsh environment [1,16]. Their cell envelopes are generally more rigid than the cell envelopes of other microorganisms or higher plants. It was reported that the tensile strength of the microalgal cell wall can be up to 9.5 MPa, which is about three times higher than that of carrot, *Daucus carota* [13]. These complex cell walls are typically tri-layered structures with high mechanical strength and chemical resistance, composed of: polysaccharides, such as cellulose, pectin, mannose, xylan; minerals, namely calcium or silicates; as well as proteins, such as glycoproteins [27,28]. The cell walls of most species of microalgae contain a relatively large proportion of cellulose, conferring structural stability