**Chapter 1. Algal polysaccharides: innovative extraction technologies, health benefits and industrial applications**

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**Abstract**

Microalgae are largely exploited due to their rich composition in high-value compounds such as carbohydrates. Algal polysaccharides and oligosaccharides offer enormous potential industrial applications due to their wide range of biological activities. The production and chemical structure of microalgal carbohydrates will vary depending on the species or strains and the culture conditions (i.e. temperature, pH and light). Moreover, microalgae are able to accumulate and/or excrete intra- and extra-cellular carbohydrates. Due to the wide heterogeneity of these compounds, the extraction and purification processes are challenging stages in the downstream processing of microalgal polysaccharides. This chapter focuses on the extraction and purification approaches to obtain carbohydrates from microalgae together with the biological activities and potential industrial applications of these compounds.

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**1. Introduction**

There is an increased interest on high-value products from algae due to the wide variety of compounds (i.e. proteins, carbohydrates and pigments) with potential applications in food/feed, cosmeceutical and pharmaceutical industries (Deniz, Garcia-Vaquero, and Imamoglu 2018, García‐Vaquero 2019, Garcia-Vaquero, Lopez-Alonso, and Hayes 2017, Hayes et al. 2019, Miranda, Lopez-Alonso, and Garcia-Vaquero 2017). Microalgae offer certain advantages for the production and commercialisation of carbohydrates over other traditional sources of these compounds. Microalgae are capable of excreting extra-cellular polymers to the culture media at different stages during their biological cycle (i.e. sulphated and non-sulphated extra-cellular polysaccharides). Moreover, the microalgal cell wall is also rich in polysaccharides (i.e. sulphated galactan hetero-polysaccharides) and the cells produce intra-cellular carbohydrates involved in different cellular processes (i.e. starch - amylose and amylopectin - and other sulphated compounds) (Arad and Levy-Ontman 2010, Singh, Kate, and Banerjee 2005, Williams and Laurens 2010). The chemical composition of microalgal polysaccharides (degree of sulphation, monosaccharide composition and linkages, type and number of chains) is influenced by the algae species and strain (Raposo, Morais, and Morais 2014a). Also, the culture conditions of microalgae (i.e. nutrients, light and temperature conditions) could be optimised to increase the production of the polysaccharides and other high-value compounds (du Plooy et al. 2015, Guiheneuf and Stengel 2015). For instance, the addition of glyoxylate to the media increased the cells’ metabolism and excretion of intra- and extra-cellular polysaccharides (Liu et al. 2010).

Due to the wide heterogeneity and industrial potential of carbohydrates, the extraction and purification strategies used to obtain these compounds from marine organisms, such as seaweed and microalgae, have recently gained a great deal of attention in the scientific community, aiming to achieve high yields of compounds while preserving intact their biological properties (Michalak and Chojnacka 2014, Garcia-Vaquero et al. 2017, Garcia-Vaquero et al. 2018, Garcia-Vaquero and Hayes 2016). This chapter summarises the current methodological approaches used to extract and purify carbohydrates from microalgae and the prospects for the industrial exploitation of microalgal polysaccharides.

**2. Extraction of microalgal polysaccharides**

Extraction techniques of carbohydrates should ideally achieve high yields of compounds, preserve the nature of the products and co-products, minimise energy consumption, generate minimum waste and be suitable for scaling-up the process to industry (Michalak and Chojnacka 2014, Garcia-Vaquero et al. 2018). Microalgae are capable of excreting extra-cellular polymers and accumulate cellular carbohydrates (cell wall and intra-cellular compounds). There are similarities, but also substantial differences, between the extraction methodologies described for extra-cellular and cellular compounds. Extra-cellular polysaccharides are released to the media during the culture of algae, and thus, the first steps in the extraction process include the separation or harvest of the algal biomass followed by the extraction techniques of choice. The remaining polysaccharides (cell wall and intra-cellular polysaccharides) require further extraction steps to achieve the desired algal extracts such as preparation and pre-treatments of the microalgal biomass, cell wall disruption techniques and precipitation of the carbohydrates of interest.

**2.1. Extraction of extra-cellular polysaccharides**

Extra-cellular polysaccharides show great potential due to the wide variety of applications and the easy and environmentally friendly production of these compounds (Öner 2013). The production of extra-cellular polysaccharides was reported in several organisms including fungi, yeasts (Duan et al. 2008, Zou, Sun, and Guo 2006) and several microalgae species such as *Porphyridium* and *Chlorella* (Guzman‐Murillo and Ascencio 2000).

The methods used to obtain extra-cellular polysaccharides from microalgae in the recent literature are summarised in table 1.

*INSERT TABLE 1 HERE*

In general, most studies include one or two steps of centrifugation and/or filtration to remove the algal biomass from the media containing the polysaccharides of interest. The wide majority of researches in the recent literature applied solvents at low temperatures to concentrate the carbohydrates without damaging their properties (Guzman‐Murillo and Ascencio 2000, Parikh and Madamwar 2006, de Jesus Raposo, de Morais, and de Morais 2014), followed by one or several precipitation steps with cationic surfactants including cetyltrimethylammonium bromide (Bae et al. 2006, Geresh et al. 2009, Yim et al. 2004) or cetylpyridinium chloride (Guzman‐Murillo and Ascencio 2000); organic solvents such as ethanol (Bae et al. 2006, Casano et al. 2015, Díaz Bayona and Garcés 2014, Magaletti et al. 2004, Pletikapic et al. 2011, Urbani et al. 2005, Yim et al. 2004, Yim et al. 2007, de Jesus Raposo, de Morais, and de Morais 2014), methanol (Mishra and Jha 2009), acetone (Parikh and Madamwar 2006) ether (Geresh et al. 2009); or combination of several of these solvents in different proportions (Geresh et al. 2009, Guzman‐Murillo and Ascencio 2000). After these solvent precipitation steps, the extracts containing polysaccharides were dialysed to remove salts or small components followed by a lyophilisation step to preserve the compounds (see multiple references on table 1).

Novel technologies are being used for extracting or fractionating carbohydrates. Membrane filtration techniques have been successfully to fractionate multiple compounds from milk (Brans et al. 2004), bacteria (Delattre et al. 2005) or plants (Wan, Prudente, and Sathivel 2012). More recently, membrane techniques have been used to extract and purify extra-cellular polysaccharides from a wide variety of microalgae species such as *Amphora* sp., *Ankistrodesmus angustus*, *Phaeodactylum tricornutum*, *Graesiella emersonii*, *Graesiella vacuolata* and *Porphyridium cruentum* (Chen et al. 2011, Marcati et al. 2014, Mezhoud et al. 2014, Patel et al. 2013, Zhang and Santschi 2009).

Filtration techniques seem well suited for initial extraction and purification steps at industrial scale as they can be automatized and permit to treat large volumes of samples (Patel et al. 2013). However, the widespread application of membranes have been hindered due to excessive membrane fouling which could result in reduced performance, severe flux decline, high energy consumption and frequent membrane cleaning or replacement (Feng et al. 2009). Recent studies focused on achieving a better understanding of anti-fouling agents (Feng et al. 2009) and other strategies to reduce fouling. For example the addition of an initial high-molecular weight cut-off membrane before the ultrafiltration step reduces the fouling of the membranes when extracting bacterial oligosaccharides (Mellal et al. 2008).

In the case of extra-cellular polysaccharides from *Porphyridium cruentum*, the tangential flow filtration (diafiltration) with a 300 kDa membrane showed better extraction, purification and desalting efficiency of high molecular weight extra-cellular polysaccharides than other methods such as dialysis and solvent-precipitation with methanol, ethanol and isopropanol (Patel et al. 2013). However, a significant proportion of cell-attached polymers were not extracted by this method. The application of a two-step membrane process with ultrafiltration and diafiltration through 300 kDa molecular weight cut-off, followed by ultrafiltration and diafiltration with a 10 kDa membrane, showed to be highly efficient for extracting high and low molecular weight extra-cellular polysaccharides and other molecules such as phycoerythrin (Marcati et al. 2014).

Other novel extraction techniques to generate polysaccharide extracts, such as ultrasonication and microwave technologies are used to breakdown extra-cellular polysaccharides, but not with the purposes of extraction (Sun et al. 2009, Sun, Wang, and Zhou 2012). Ultrasonication and microwave technologies could be important tools during the development of pharmaceutical products from polysaccharides. Most products from marine origin with pharmaceutical and cosmeceutical properties currently on the market and approved by the Food and Drug Administration or the European Medicines Agency, are modifications of the natural molecules obtained during the processes of optimisation and drug development (Martins et al. 2014).

**2.2. Extraction of cellular polysaccharides**

Less information is available concerning the extraction of cellular polysaccharides from microalgae. Traditionally these polymers were not extracted and purified to obtain high-value products. The majority of the scientific literature related to cellular polysaccharides focus on general extraction of carbohydrates or fermentation of the biomass from oil extraction to produce bioethanol (Behera et al. 2014). Due to the low lignin and hemicellulose contents, microalgae have been considered a suitable source of polysaccharides for bioethanol production as an alternative to conventional crops such as corn and soy bean (Behera et al. 2014, Chaudhary et al. 2014). Several microalgae species have shown promising results in this field. For example, *Chlorococcum* produced high yields of bioethanol (Harun and Danquah 2011, Harun et al. 2011), at levels comparable or even higher than those obtained from traditional crops like rice straw or sugar cane (Behera et al. 2014).

The downstream processing steps required to produce microalgal cellular carbohydrates include energy and cost intensive steps such as harvesting and concentration of the biomass such as filtration, flocculation, centrifugation and sonication (Liang 2015, Vandamme, Foubert, and Muylaert 2013) followed by other processes to dry the biomass using spray-, drum-, freeze- and sun-drying techniques (Behera et al. 2014). Some authors avoid the extra-energy consumption to dry the biomass if there are no preservation issues or the extraction processes are not affected by moisture. The extraction process normally starts with different pre-treatments to eliminate pigments, lipids or proteins (Casano et al. 2015, Cheng, Labavitch, and VanderGheynst 2015, Sadovskaya et al. 2014) followed by different methods designed to breakdown the cell walls and precipitate the polysaccharides of interest for further purification and characterisation (see table 2).

*INSERT TABLE 2 HERE*

The breakdown of the cell walls is normally achieved by conventional strategies such as solubilisation of biomass in water (Casano et al. 2015, Lee et al. 2000, Lee et al. 1998, Mader et al. 2016) or solutions of CaCl2 (Sadovskaya et al. 2014) and ethanol (Balavigneswaran et al. 2013, Pugh et al. 2001). Recently, new technologies have been used for this purpose such as sonication (alone or in combination with heat treatment) (Guzman et al. 2003, Jo et al. 2010, Sun et al. 2016, Sun et al. 2014) and subcritical water extraction (Chakraborty et al. 2012). Other innovative extraction processes include the use of enzymes – i.e. proteases (ProtamexTM) (Jo et al. 2010) and α-amylase and amyloglucosidase (Cheng, Labavitch, and VanderGheynst 2015, Cheng et al. 2011, Fu et al. 2010). Although promising, the enzymatic treatments do not have industrial applications to date due to the high cost and time required by these processes (Michalak and Chojnacka 2014).

After breaking down the cell walls, the dissolved polymers can be precipitated by using membrane filtration systems (Guzman et al. 2003, Jo et al. 2010) and different solvents such as ethanol (Balavigneswaran et al. 2013, Casano et al. 2015, Chakraborty et al. 2012, Sun et al. 2016, Sun et al. 2014), methanol (Jo et al. 2010), acetone or ether (Sun et al. 2014). Independently of the use or not of pre-treatments, some authors also describe techniques to remove undesired compounds (mainly lipids and proteins) at this stage i.e. trichloroacetic acid (Lee et al. 2000, Lee et al. 1998, Mader et al. 2016), chloroform and butanol (Sadovskaya et al. 2014) and octanol in chloroform (Sun et al. 2014).

**3. Purification of microalgal polysaccharides**

After the extraction procedures, extra-cellular or cellular polysaccharides could go through one or several purification steps that do not differ to those described for other carbohydrates. The purification techniques commonly used in microalgal polysaccharides include the application of different solvents (hexane, ethyl acetate, acetone, ethanol, petroleum ether, methanol and water) to separate the molecules with respects to their polarity (Challouf et al. 2011, Jo et al. 2010). Other chromatographic techniques include ion-exchange chromatography (Huheihel et al. 2002, Huleihel et al. 2001, Guzman et al. 2003, Sadovskaya et al. 2014, Sun et al. 2014) and gel permeation chromatography (Bae et al. 2006, Casano et al. 2015, Feng et al. 2009, Huleihel et al. 2001, Magaletti et al. 2004, Yim et al. 2004, Guzman et al. 2003). The use of chromatographic and non-chromatographic techniques to purify and characterise carbohydrates was recently reviewed in detail by Garcia-Vaquero (2019).

**4. Industrial applications and prospects of microalgal polysaccharides**

Microalgae are traditionally commercialised as full dried biomass mainly for human food and animal feed applications in the form of powders, tablets, capsules or liquids (Spolaore et al. 2006). The main microalgae species commercialised as whole dried biomass are *Spirulina* sp. and *Chlorella* sp. with an approximate turnover of 80 million USD per year (Vigani et al. 2015). Other high-value products obtained from microalgae are currently in the market, with important applications in the food, pharmaceutical, cosmeceutical and energy industries (Ventura et al. 2018). The high-value compounds commercialised from microalgae include pigments (i.e. asthaxanthin, ß-carotene), PUFA (mainly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)) and proteins (phycobiliproteins) (Ventura et al. 2018).

Microalgae are also a promising source of other valuable compounds such as polysaccharides. For instance, extra-cellular and sulphated polysaccharides extracted from *Porphyridium*, *Chlorella* and *Spirulina* sp. namely alguronic acid are used as cosmeceutical, nutraceutical and pharmaceutical (Borowitzka 2013, Laurienzo 2010, Raposo, de Morais, and Bernardo de Morais 2013). Recently, microalgal polysaccharides have dragged the attention of the scientific community due to the wide range of biological properties of these compounds (see tables 1 and 2). Several bioactivities from microalgal polysaccharides include anti-viral, anti-bacterial, antioxidant, anti-inflammatory, immunomodulatory, anti-tumour, anti-lipidemic, anti-glycemic, anti-coagulant, anti-thrombotic, bio-lubricant and anti-adhesive properties (Raposo, Morais, and RMSC 2014a). Moreover, extra-cellular polysaccharides produced by cyanobacteria have also been used as soil conditioners, improving the water holding capacity of the soil and the detoxification of heavy metals/radionuclides and removal of solid matter from contaminated water (Bender and Phillips 2004). Some extra-cellular polysaccharides produced by marine microorganisms are currently in the cosmeceutical market with great success. For example, extra-cellular polysaccharides from *Alteromonas macleodii* (Abyssine®) and glycoproteins from *Pseudoalteromonas* sp. (SeaCode®) (Martins et al. 2014). However, the main producers of extra-cellular polysaccharides currently in the market are bacteria from the species *Xanthomonas*, *Leuconostoc*, *Sphingomonas* and *Alcaligenes* that produce xanthan, dextran, gellan and curdlan (Öner 2013).

Despite the great potential of microalgae for the generation of high-value compounds, there are certain challenges that this industry has to address to increase the presence of microalgal products in the market, such as the strong presence in the market of chemically synthesised compounds. The successful establishment of microalgal products will depend not only on the aptitude of the different microalgae species to produce high-value compounds, but in reducing the cost of production and downstream processing of the biomass, designing new products and creating new markets (de la Jara et al. 2016).

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**Table 1.** Summary of the extraction methods used to obtain extra-cellular polysaccharides from microalgae and the potential use or bioactivity of these compounds.

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| --- | --- | --- | --- |
| **Microalgae sp.** | **Extraction method** | **Potential use or bioactivity** | **References** |
| *Porphyridium cruentum*, *Chaetoceros* sp., *Chlorella autotrophica, Chlorella capsulate, Chlorella* sp., *Dunalliela tertiolecta, Isochrysis galbana, Isochrysis* sp., *Nannochloropsis oculata, Phaeodactylum tricornutum, Rodhosorus marinus, Tetraselmis* sp., *Tetraselmis suecica, Botrycoccus sudeticus, Botryococcus braunii; Chlamydomonas Mexicana; Chlorococcum oleofaciens, Dysmorphococcus globosus, Hormotilopsis gelatinosa, Neochloris oleoabundans* and *Ochromonas danica* | Centrifugation followed by filtration (0.45 µm), heating (40°C, 1h) and precipitation with cetylpyridinium chloride. The precipitate collected by centrifugation was precipitated with CaCl2, and the pellet washed with ethanol, ethanol:ether and ether. | Anti-bacterial (inhibition of the cytoadhesion process of *Helicobacter pylori* to HeLa S3 cells). | Guzman‐Murillo and Ascencio (2000) |
| *Gyrodinium impudicum* KG03 | Centrifugation followed by ethanol precipitation, cetyltrimethylammonium bromide re-precipitation. The pellet collected was re-suspended in NaCl, precipitated with ethanol and dialysed. | Anti-viral (encephalomyocarditis virus) and anti-tumour. | Bae et al. (2006), Yim et al. (2004) |
| *Porphyridium* sp. | Centrifugation followed by precipitation of the supernatant with NaOH and different solvents (HCl, NaCl, ethanol, cetyltrimethylammonium bromide and acetone). | - | Geresh et al. (2009) |
| *Amphora* sp., *Ankistrodesmus angustus* and *Phaeodactylum tricornutum* | Centrifugation to separate the cells followed by filtration, crossed-flow ultrafiltration and stirred cell diafiltration of the supernatant. | - | Chen et al. (2011) |
| *Porphyridium cruentum* | Diafiltration (tangential flow filtration with 300 kDa membrane). | - | Patel et al. (2013) |
| *Porphyridium cruentum* | 2 step concentration-diafiltration with different molecular weight cut-off membranes. | - | Marcati et al. (2014) |
| *Trebouxia* sp. from lichen *Ramalina farinacea* | Centrifugation followed by ethanol precipitation 2 times of the supernatant. | - | Casano et al. (2015) |

**Table 2.** Summary of the extraction methods used to obtain cellular polysaccharides from microalgae and the potential use or bioactivity of these compounds.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Microalgae sp.** | **Pre-treatment** | **Extraction method** | **Potential use or bioactivity** | **References** |
| *Tetraselmis suecica* | - | Dried algae was suspended in water, sonicated and incubated with a protease enzyme (50°C, 3h). After centrifugation, the supernatant was ultrafiltrated. | Anti-inflammatory. | Jo et al. (2010) |
| *Chlorella sorokiniana* | - | Subcritical water extraction (2 times at 160°C and 180°C) followed by ethanol precipitation and lyophilisation of supernatant. | - | Chakraborty et al. (2012) |
| *Isocrysis galbana* | Dried biomass with 70% ethanol (85°C, 2 h, 3 times) | Ethanol precipitation, filtration, acetone:chloroform washes (3 times), hot water extraction and ethanol precipitation. | Antioxidant (hydrogen peroxide, DPPH and superoxide anion). | Balavigneswaran et al. (2013) |
| *Chlorella vulgaris, Chlorella sorokiniana, Chlorella minutissima* and *Chlorella variabilis* | Dried biomass treated with chloroform:methanol (3 times). Precipitate washed in NaCl to remove proteins followed by acetone (85°C, 3 times) to remove starch. | Pre-treated pellet was incubated with α-amylase and amyloglucosidase (37°C, overnight) and the cell walls were collected. | - | Cheng, Labavitch, and VanderGheynst (2015), Cheng et al. (2011) |
| *Isochrysis galbana* | - | Dried algae were re-suspended in water, sonicated and heated (70°C, 180 min). Supernatant was concentrated with ethanol. Pellet was washed with ethanol, acetone and ether and treated with Sevag method (remove proteins) and dialysis. | Antioxidant. | Sun et al. (2014) |
| *Trebouxia* sp. from lichen *Ramalina farinacea* | Dried biomass was pressure-treated (1200 psi). | Pellet was re-dissolved and extracted with ethanol, chloroform:methanol and acetone (different times and temperatures, followed by boiling water and ethanol precipitation. | - | Casano et al. (2015) |
| *Pavlova viridis* | - | Dried algae re-suspended in water, sonicated (65°C). Supernatant was concentrated, treated with trichloroacetic acid (precipitate proteins) and supernatant dialysed and precipitated with ethanol several times. | Immunomodulation and anti-tumour. | Sun et al. (2016) |
| *Isochrysis galbana* | Dried algae mixed with ethanol (different % and temperatures, 2 times) | Pre-treated pellet was extracted with CaCl2 (boiling temperature, 3 times). Pooled supernatants were ethanol precipitated and pellet washed with chloroform (remove proteins and lipophilic materials) and dialysed. | Anti-tumour activity. | Sadovskaya et al. (2014) |

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