Cell disruption technologies

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6.1 Introduction

Algal cell walls separate the inside cell content from the environment to protect the cell against desiccation, pathogens, and predators while still allowing exchange of compounds. Toward application of algae biomass as a sustainable resource, disruption of this cell wall (=cell disruption) is an essential pretreatment step to maximize product recovery in downstream processes of the algae biorefinery. Also for direct use of algae in feed or food, cell rupture is required to increase the bioavailability of algae constituents. Depending on the cell wall structure, the size, and the shape of algae, cell disruption can be challenging. A variety of cell disruption methods is currently available, and new approaches are being elaborated in parallel. Since downstream processing is responsible for a large part of the operational costs in the whole production chain, cell disruption technologies should be low cost and energy efficient and result preferably in high product quality. This chapter provides information on cell wall types and gives an overview of physical-mechanical and (bio-)chemical cell disruption technologies with attention to development stage, energy efficiency, product quality, costs, emerging approaches, and applicability on large scale.

6.2 Cell wall types in various groups of microalgae and cyanobacteria

The cell wall composition and architecture of algae and cyanobacteria are highly variable ranging from tiny membranes to multilayered complex structures. Despite the importance of algal cell wall properties in biotechnology, little structural information is available for most species (Scholz et al., 2014). Based on the complexity of surface structures, four cell types could be distinguished (Barsanti and Gualtieri, 2006; Lee, 2008) (Fig. 6.1).

A *simple cell membrane* (Fig. 6.1, Type 1) is present in short-lived stages (e.g., gametes), chrysophytes, raphidophytes, green algae *Dunaliella*, and haptophytes *Isochrysis*. It consists of a lipid bilayer with integrated and peripheral proteins. Sometimes a cap of glycolipids and glycoproteins envelops the outer surface of cell membrane. *Cell membranes with additional extracellular material* are known in cyanobacteria and many groups of algae, including palmelloid phases. It is the most diverse cell wall type that includes various membrane-associated structures (cell wall,

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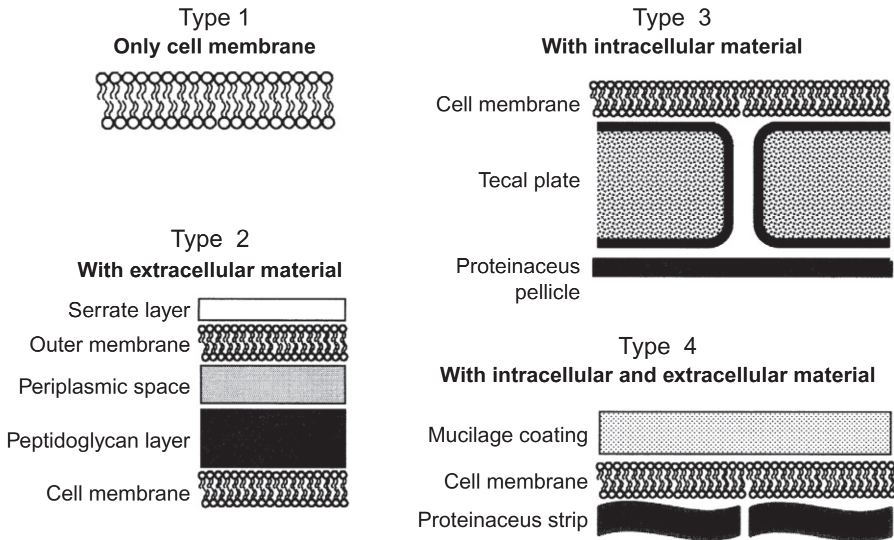

Fig. 6.1 Schematic view of cyanobacteria and algae cell wall types.
Modified from Lee, R.E., 2008. Phycology. Cambridge University Press.

mucilage and sheaths, scales, frustules, lorica, skeleton). Cell walls of prokaryotic and eukaryotic algae are rigid, homogeneous, and often multilayered. The cell wall of cyanobacteria is a six-layered structure (Fig. 6.1, Type 2) with a rigid peptidoglycan layer overlaying the inner cell membrane and tightly connected with the outer membrane of the wall containing muramic acid on top. Eukaryotic algal cell walls are formed outside the cell membrane and are generally made up of two components, a microfibrillar framework embedded in an amorphous mucilaginous material composed of polysaccharides, lipids, and proteins. Mucilages and sheaths serve as wall joint material and support movement or protect the cell. The gelatinous cover mainly contains exopolysaccharides. Scales are organic or inorganic surface structures individually scattered on the surface, arranged in a specific pattern or forming an envelope. The frustule of diatoms is an ornate cell membrane made of amorphous hydrated silica. Extracellular organic coats envelop the plasma membrane under the siliceous frustule. Lorica is a specific structure from fine cellulose or chitin fibrils or imbricate scales whereas siliceous skeletons are situated outside the plasma membrane and contain a three-dimensional structure. Dinoflagellates have cell membranes with *additional intracellular material in vesicles* (Fig. 6.1, Type 3). They are termed amphiesma and comprise an outer plasmalemma overlaying a single layer of flattened vesicles. Each vesicle may lack or contain one to several cellulosic thecal plates. The layer consists primarily of cellulose, sometimes with a dinosporin. *Cell membranes with intracellular and extracellular material* are characteristic for euglenophytes and cryptophytes (Fig. 6.1, Type 4). A pellicle of Euglenophyta contains four components: plasma membrane, proteinaceous strips, microtubules, and tubular cisternae of endoplasmic reticulum. The plasma membrane has an external mucilage sheath. The

periplast of cryptophyte also consists of components present on both sides of the membrane. The inner component comprises proteins and may consist of fibril material, a single sheet or multiple plates. The outer component may have plates, heptagonal scales, mucilage, or combinations of these.

Although thousands of microalgae species exist, only hundreds of these have been studied and used for various biotechnological applications. Among these are cyanobacteria—*Aphanizomenon*, *Arthrospira*; green algae—*Dunaliella*, *Haematococcus*, *Scenedesmus*/*Desmodesmus*, *Chlorella*, *Tetraselmis*; red algae—*Porphyridium*; diatoms—*Phaeodactylum*, *ochrophytes*, *Nannochloropsis*; and haptophytes—*Isochrysis*. Recently, mainly *Spirulina*, *Chlorella*, *Haematococcus*, and *Dunaliella* have been grown commercially on a large scale. The cell wall structures of species most often used in biotechnology are given in Fig. 6.2. *Dunaliella* and *Isochrysis* cells are naked (Type 1) and therefore fragile for disruption whereas the others have more complex Type 2 cell wall structures that are more challenging for the extraction of intracellular products.

More in detail, the structure of *Spirulina* cell walls has four distinct layers and is of gram-negative bacteria type (Van Eykelenburg, 1977; Berner, 1993). The peptidoglycan layer, also known as murein, provides rigidity and is located between two fibrillar layers. The outer membrane is tightly connected with the peptidoglycan layer and is covered with a sheath of acidic polysaccharides (Tomaselli, 1997). The cell wall of

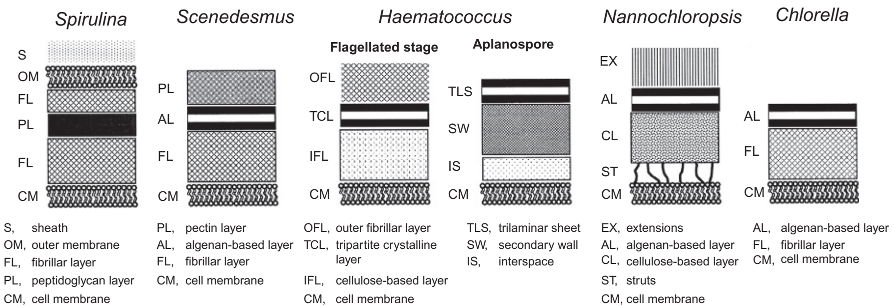

Fig. 6.2 Schematic view of cell wall structures of biotechnologically important species. Modified from Berner, T., 1993. Ultrastructure of Microalgae. CRC Press; Bisalputra, T., Weier, T.E., 1963. The cell wall of *Scenedesmus quadricauda*. Am. J. Bot. 50 (10), 1011–1019; Gerken, H.G., Donohoe, B., Knoshaug, E.P., 2013. Enzymatic cell wall degradation of *Chlorella vulgaris* and other microalgae for biofuels production. Planta 237, 239–253; Hagen, C., Siegmund, S., Braune, W., 2002. Ultrastructural and chemical changes in the cell wall of *Haematococcus pluvialis* (Volvocales, Chlorophyta) during aplanospore formation. Eur. J. Phycol. 37(2), 217–226; Montsant, A., Zarka, A., Boussiba, S., 2001. Presence of a nonhydrolyzable biopolymer in the cell wall of vegetative cells and astaxanthin-rich cysts of *Haematococcus pluvialis* (Chlorophyceae). Mar. Biotechnol. 3, 515–521; Scholz, M.J., Weiss, T.L., Jinkerson, R.E., Jing, J., Roth, R., Goodenough, U., Posewitz, M.C., Gerken, H.G., 2014. Ultrastructure and composition of the *Nannochloropsis gaditana* cell wall. Eukaryot. Cell 13(11), 1450–1464; Van Eykelenburg, C., 1977. On the morphology and ultrastructure of the cell wall of *Spirulina platensis*. Antonie Van Leeuwenhoek 43, 89–99.

Scenedesmus consists of three layers: an inner cellulosic layer delimiting individual cells, a thin middle algaenan-based layer, and an outer pectic layer joining the cells into coenobium (Bisalputra and Weier, 1963). Based on scanning microscopy, pectic layers consist of a hexagonal network of electron-dense material on the surface, and a system of tubules radiating out from the middle layer. *Haematococcus,* a motile cell, has a wide distinct gelatinous multilayered extracellular matrix made up of interlocking fibers, granular and crystalline elements (Hagen et al., 2002). The tripartite crystalline layer interjects between inner layer composed of a loose net of fibrous-granular structures and the outer fibrous stratum. Both fibrous layers probably form a continuous network. In the aplanospore, the interspace separates plasmalemma and the secondary wall, which was assumed to be composed of granulose nonfibrillar mannan. The outer wall layer contains highly resistant algaenan at eight times higher concentrations compared to vegetative cells (Montsant et al., 2001). For *Nannochloropsis*, the inner layer of the cell wall is porous with a delicate fibrous substructure and struts connecting this layer to the plasma membrane (Scholz et al., 2014). This layer is primarily composed of cellulose and glucose; amino acids represent an integral cell wall constituent. Small amounts of other sugars (rhamnose, mannose, ribose, xylose, fucose, and galactose) may be present in some *Nannochloropsis* species (Brown, 1991). Algaenan layers comprise a thin trilaminar sheath in the cell periphery. Extensions of unknown composition protrude from the outer surface layer in *Nannochloropsis gaditana*. The cell wall structure of *Chlorella* varies among species and strains, and also depends on growth conditions. Generally, the inner cell wall layer is composed a rigid microfibrillar structure embedded into a continuous matrix. The layer has a high cellulose content (Rodrigues and da Silva Bon, 2011), and chitin-like glycan is a predominant amino sugar in the rigid cell wall. The rigid wall components embedded within a more plastic polymeric matrix are composed of uronic acids, rhamnose, arabinose, fucose, xylose, mannose, galactose, glucose, and pectin (Gerken et al., 2013). In addition, the outer cell wall of different species may include a trilaminar algaenan or form a thin homogeneous monolayer. Most species used in biotechnology have an algaenan layer (Fig. 6.2). Algaenans are highly resistant aliphatic polymers (Scholz et al., 2014 and references therein) and therefore expensive techniques are required for cell wall fracturing. Algaenan is known in vegetative cells of some *Scenedesmus*, *Chlorella*, and *Nannochloropsis* species; *Botryococcus* colonies; and cysts of *Chlamydomonas*, *Haematococcus,* and *Polytomella*. Algaenans of *Nannochloropsis* are comprised of straight-chain (~C30), highly saturated aliphatic compounds cross-linked by ether bonds at terminal and one or two midchain positions. Whereas in *Tetraedron minimum*, *Scenedesmus communis*, and *Pediastrum boryanum*, it probably consists of very long-chain (up to C120) monomeric (di)carboxylic acids; in *Botryococcus braunii*, it comprises polyacetals that are cross-linked or not linked by terpene epoxides.

6.3 Physical methods for cell wall disruption

Numerous cell disruption techniques have been tested in an attempt to find the most efficient device to increase the extraction yield of intracellular products from microalgae. Lower product contamination and less dependence on microalgae species are

discussed among the advantages of physical-mechanical disruption methods. However, when compared to chemical and biological pretreatments, they require more sophisticated equipment and higher energy inputs for processing whereas the generated heat can damage the end products. Recently, the most used methods have been reviewed, for instance, by Al hattab and Ghaly (2015), Günerken et al. (2015), Halim et al. (2012a), Kim et al. (2013), Kumar et al. (2015), Lee et al. (2012), McMillan et al. (2013), Mubarak et al. (2015), and Show et al. (2015). Physical pretreatment is classified based on the nature of the forces causing cell wall disruption and could be subdivided into *thermal* and *mechanical* (solid and liquid share forces, waves, and currents) methods.

During *thermal pretreatment*, microalgae cells are disrupted using heat/frost and can be subdivided into high (>100°C), mild (50–100°C), and freezing temperature methods. *The freeze-fracture* method involves a series of freezing-defrost cycles, and cell disruption is achieved due to ice crystal formation and cell expansion upon thawing. During *freeze-drying* (lyophilization) a pressure of about 1 kPa and temperatures of less than −40°C are applied to slowly frozen algae samples. Cell walls become more porous due to formation of large ice crystals during slow freezing, and these crystals sublime in the lyophilization process. *Mild temperatures* can be applied to stimulate the activity of thermophilic and hyperthermophilic bacteria to disrupt the cell wall during a biological pretreatment. *High-temperature* methods are generally applied in biocrude oil and biogas production and are among the most performant cell wall disruption methods. The use of steam improves heat transfer, and less energy is required to break the hydrogen bonds, provoking structural changes. The pretreatment is unspecific, promoting reactions on the different components of microalgae. Carbohydrates are depolymerized into monomers and proteins into organic and carbonic acids, amides, and ammonia. The previously mentioned products have individual commercial applications, but above 300°C, biocrude is the preferred end product. Under these conditions, lipids can release fatty acids, which are able to decompose into hydrocarbons (Patel et al., 2016). The main operational parameters are the type of microalgae, temperature, pressure, and time (Passos et al., 2015b). Other advantages of thermal pretreatments are cost-effectiveness, wide availability, and no use of chemicals.

Combined pressure and temperature pretreatment can be divided in two groups according to the rate of pressure release: high temperature or hydrothermal pretreatment (slow depressurization) and steam explosion (sudden depressurization). *Hydrothermal pretreatments* have exposure times of 5–30 min, temperatures above 100°C, and elevated pressures (<220 bars) in an autoclave. During *steam explosion*, biomass remains under saturated vapor pressure at high temperature (140–180°C) and pressure for a variable time (from 5 to 60 min) (Yoo et al., 2014). The entire mixture of microalgae and steam is subsequently flashed, and the biomass is cooled down in another vessel. Cell disruption occurs due to the rapid pressure drop (Carrere et al., 2016). This pretreatment has been scaled up in wastewater treatment plants to increase biogas production from sludge. However, data are scarce in the research field of microalgae. For instance, Lorente et al. (2015) applied steam explosion (120–150°C at 2–4.7 bar for 5 min) for the extraction of lipids and sugar from single cultures of

N. gaditana. After pretreatment at the optimal conditions (150°C), 44% of the 13.5% of total carbohydrates present on a dry ash-free basis was extracted, and the extractable lipids were found to increase from 9.8% to 18.2%.

Mechanical pretreatment directly breaks cell wall components through a physical force. Bead mill, high-pressure homogenization, and ultrasonication are the most widely used mechanical methods for microalgal cell disruption. *Bead milling* is one of the most effective techniques and uses kinetic energy to force small beads (glass, ceramic, plastic, or steel) to collide with each other and the algae cells. Agitated beads are more efficient than shaking vessels. Alternatively, various *press* configurations (screw, expeller, piston, etc.) are used. The expeller press uses a mechanical force to compress microalgae cells and to squeeze content out of the cell. The *high-speed homogenizer* combines hydrodynamic cavitation generated by stirring at high rpm and mechanical forces such as shear for cell wall disruption. *High-pressure homogenization* or French press is one of the earliest techniques developed to disrupt algal cells. Microalgal concentrate is pumped through a narrow orifice (~80–200 μm) in a valve under high pressure (138–400 MPa), and the suspension is then released into a low-pressure chamber. Cell wall disruption occurs due to high-pressure impingement of accelerated cellular jet on the stable valve surface and due to a pressure drop-induced shear stress when the cell passes from valve to chamber. The designed various valve-seat configurations allow maximization of cell disruption efficiency and minimization of valve seat damage due to cavitation.

Microwaves have been broadly applied as an alternative thermal pretreatment of biomass feedstocks, including lignocellulosic, microalgae, and macroalgae biomass (Ju et al., 2016). The frequency ranges from 0.3 to 300 GHz, of which 2450 MHz waves are typically used for microalgae cell wall disruption (Günerken et al., 2015). The mechanism is based on the interaction of electromagnetic waves with dielectric and polar molecules provoking local heating and an internal pressure increase. High concentrations of biomass benefit the specific energy consumption by increasing the energy directed to biomass. Apart from biomass concentration, pretreatment time and power of microwaves are the main operation parameters. Passos et al. (2015a) used microwaves to increase the soluble fraction of organic matter, proteins, carbohydrates, and lipids by a factor of 8, 18, 12, and nearly 2, respectively, compared to the untreated biomass. Silva et al. (2014) applied a microwave pretreatment (400 W, 4 min) on mixed culture biomass of *Chlorophyceae*, *Cyanophyceae*, *Euglenophyceae*, and *Bacillariophyceae* and reported a remarkably higher lipid extraction (33.7%) compared to the untreated sample (4.8%). Advantages of the microwave technology include effectiveness, robustness, and easy scale-up, but effectiveness depends largely on the type of microalgae (Günerken et al., 2015; Passos et al., 2015b).

Ultrasound waves induce alternations of high- and low-pressure cycles in the liquid. Microbubbles created during low-pressure cycles ultimately implode in high-pressure cycles and produce local shock waves (cavitation) creating (1) acoustic vibrations, (2) extreme temperature peaks, and (3) thermolysis of water around the bubbles forming highly reactive free radicals. This pretreatment has been tested for

cell disruption at different frequencies (20 kHz, 40 kHz, and 1 MHz), although frequencies applied at a large scale are lower due to energy consumption concerns (18, 20, 24, and 30 kHz). Ultrasonic cavitation at low (18–40 kHz) frequency is much stronger than at high frequency (400–800 kHz). Nevertheless, its efficiency depends on the microalgae species (shape, size, intracellular structure), operational conditions (temperature, time, power, number of cycles), and biomass concentration (Günerken et al., 2015; Passos et al., 2015b). Ultrasound microalgae pretreatment has been mainly applied for biodiesel, bioethanol, and biogas production and has been proven to adequately break algal cells in low concentration biomass suspension. Passos et al. (2015a) reported that ultrasound pretreatment (20 kHz, 30 min, 26.7 MJ/kg TS) increased the soluble fraction of organic matter, proteins, carbohydrates, and lipids 7-, 12-, 9-, and 3-fold compared to untreated samples. Likewise, Silva et al. (2014) applied ultrasounds (40 kHz, 60 min) on a mixed microalgae culture composed of *Chlorophyceae*, *Cyanophyceae*, *Euglenophyceae*, and *Bacillariophyceae*, obtaining lipid extraction yields of 13.3%. However, the overall heat production degraded a fraction of the proteins and generated metabolites, which were identified as the main drawback of this pretreatment.

Pulse electric field is a promising novel technique for cell disruption (Joannes et al., 2015). The microalgal suspension is placed between two electrodes and a pulsed electric field is applied to cause electroporation to increase the permeability of the cell wall. This can induce reversible or irreversible damage depending on electroporation strength.

Table 6.1 summarizes the advantages and limitations reported for the above described disruption technologies. Generally, mechanical methods are inconvenient, long lasting, difficult to apply on large scale and not sufficiently effective, while the thermal methods are energy intensive and costly.

Table 6.1 Advantages and disadvantages of physical-mechanical cell disruption methods

Method	Advantages	Disadvantages
Freeze-fracture	Extraction of fragile compounds	Time-consuming, energy intensive
Freeze-drying	Gentle extraction of fragile compounds (protein, enzymes)	High energy and time-consuming, high maintenance cost, difficult to scale up, degradation of lipids
Mild temperature	Low energy consumption, simple; can be applied on algal slurry	Time-consuming, low effectiveness for algae with complex cell wall, algae species sensitive

Continued

Table 6.1 Continued

Method	Advantages	Disadvantages
Autoclaving	Simple; can be applied on algal slurry	High energy consumption, low effectiveness for some species, scale-up difficulty, long process, degradation of thermolabile compounds
Steam explosion	Easy to scale up; can be applied on algal slurry	High energy consumption, species-specific effectiveness, degradation of some compounds
Bead milling	Simple equipment, rapid process, high disruption efficiency, easy scale-up; suitable for large-scale, low labor intensity; low operating cost; can be applied on algal slurry	High energy consumption; requires extensive cooling for thermolabile compounds, formation of very fine cell debris
Expeller Press	Simple method, efficient, application on large scale for particular products	High energy consumption, temperature rise, high maintenance cost; requires dry biomass, prolonged processing time; choking problems; species-dependent effectiveness; rigid cell wall hinder product release
High speed homogenization	Simple, effective, short contact time; can be applied on algal slurry	High energy consumption; temperature rise may lead to degradation of thermolabile compounds; species-dependent effectiveness; rigid cell wall may hinder product release; contamination with abrasive materials
High-pressure homogenization	High efficiency; does not require biomass drying; easy scale-up	High energy consumption; temperature rise may lead to degradation of thermolabile compounds; rigid cell wall may hinder product release; very fine cell debris
Microwave	Simple, rapid process; effective for robust species; easy to scale up; low operating costs; does not require dewatering of algal biomass	High energy consumption and maintenance costs, high temperature; recovery of thermolabile compounds may require cooling; lipid degradation and protein aggregation, denaturation, formation of free radicals

Table 6.1 Continued

Method	Advantages	Disadvantages
Ultrasonication	Simple, short extraction time, high reproducibility, efficient	Moderate energetic costs, temperature rise, rigid cell wall hinders product release; production of reactive hydroxyl radicals; not applicable to large-scale, sonication; energy effective in small volume
Pulsed electric field treatment	Simple, highly energetically efficient, relatively fast, easy to scale up; can be combined with other methods	High maintenance costs, temperature rise, dependence on medium composition, decomposition of fragile compounds

Based on Al hattab and Ghaly (2015), Gonçalves et al. (2013), Günerken et al. (2015), Halim et al. (2012a), Joannes et al. (2015), Kim et al. (2013), Kumar et al. (2015), Lee et al. (2012), McMillan et al. (2013), Mubarak et al. (2015), Passos et al. (2015a,b), and Show et al. (2015).

6.4 (Bio)chemical methods for cell wall disruption

Chemical cell disruption has been widely studied using a large variety of compounds such as antibiotics, chelating agents, chaotropes, detergents, solvents, oxidizing agents, acids, and alkali (Günerken et al., 2015). The main operational parameters are the type and concentration of chemical, temperature, time, biomass concentration, and microalgae species (Lam and Lee, 2015). Chemical methods can be more selective than mechanical methods since they often rely on specific interactions with cell wall constituents. Additionally, energy consumption is generally lower, cell disruption efficiency is higher, and upscaling is more simple. However, the cost of chemicals and quality of the products might reduce their benefits.

In *acid and alkaline* methods, H_2SO_4 is the most applied acid whereas NaOH is the most studied base. Treatments have been shown to be effective as pretreatment for fermentation and extraction of intercellular compounds such as lipids and pigments (Mendes-Pinto et al., 2001; Miranda et al., 2012; Nguyen et al., 2009). The reaction can be performed at ambient or elevated pressure, and temperatures above 100°C enhance reaction rates. Concentrated acid-based methods are faster but have some drawbacks such as the generation of inhibitors, equipment corrosion, difficult chemical recovery, or high operational and preservation costs. Although more time-consuming, diluted pretreatments seem therefore more advantageous for industrial applications (Günerken et al., 2015). Acid and alkaline pretreatments display low selectivity releasing multiple components, which results in a difficult separation. Denaturation of proteins can occur in alkali media and degradation of pigments in acid environments (Günerken et al., 2015).

Extraction by *ionic liquids* is another rather new cell disruption method. It has been studied for lipid extraction and ethanol production processes. The advantages of this method are its low volatility, high capacity to dissolve organic molecules, short reaction times, recovery and reuse of ionic liquid, and, depending on the type of ionic liquid, its bifunctionality as a solvent for lipid extraction as well as a catalyst for transesterification. Despite the fact that ionic liquids reveal positive results for disrupting algal biomass, their high price and toxicity are the main hurdles for implementation on a large scale. Kim et al. (2012) reported a lipid extraction increase of ~19% from dry *Chlorella vulgaris* (11.1% extractable lipids from untreated samples), working at 65°C, 18 h with three different ionic liquids. Zhou et al. (2012) released between 65 and 88 wt% of the total sugars contained in *C. vulgaris* (73.58% of total sugars) working at 105°C, 3 h with different ionic liquids and HCl (7 wt% acid).

Supercritical fluids display both liquid and gas characteristics, which offer the potential for cell disruption (Mohan et al., 2013). The interaction between fluid properties (density, diffusivity, surface tension, viscosity) and operational parameters (temperature, pressure, biomass concentration) favors the separation of desirable products (Bahadar et al., 2015). The most used supercritical fluid is CO_2, which is applied mainly for lipid extraction and, to a lesser extent, to carbohydrate release. Bahadar et al. (2015) applied a supercritical method on *C. vulgaris* (18% of total lipids), reporting a maximum lipid extraction yield of 17.7 wt% at 7000 psi, 60°C and 3 g CO_2/min for 3 h. Thana et al. (2008) reported an astaxanthin recovery of 83.8% with an extraction yield of 23 mg/g cell from *Haematococcus pluvialis* using CO_2 supercritical optimal conditions (50 MPa, 70°C, 4 h). Research on supercritical CO_2 to extract microalgae carbohydrates has recently been conducted by Harun et al. (2010), who worked at 60°C, 400 mL/min of CO_2 and obtained 60% higher ethanol concentration compared to nonpretreated biomass. Advantages of this method are the use of nontoxic solvents, the solvation capability to be fine-tuned via pressure and temperature settings, and the extensive availability of equipment. Disadvantages include high power consumption and costs and challenging upscaling.

Enzymatic hydrolysis is a biochemical cell disruption method through the conversion of carbohydrates into monomeric sugars or proteins into amino acids, resulting in cell wall disruption. The type of enzyme is the key factor related to operational costs and determines the process conditions (temperature, pH, biomass content, enzyme concentration, time, and agitation). Frequently used enzymes for enzymatic hydrolysis are cellulases, glycosidases, amylases, proteases, xylanases, peptidases, and lipases (Lam and Lee, 2015). Enzymatic hydrolysis presents advantages over the acid/alkali pretreatment such as biological specificity, high selectivity, high conversion yield, mild operating conditions, low energy requirements, low capital investment, easily scale-up, and the prevention of destructive conditions. Nevertheless, drawbacks are inhibitor production, high enzyme cost, difficult enzyme recovery, and long incubation times (Lam and Lee, 2015). Ho et al. (2013) obtained 90.4% of glucose release from *C. vulgaris* FSP-E using endoglucanase (0.65 U/mL), β-glucosidase (1.50 U/mL), and amylase (0.09 U/mL) following a 3-day incubation at 200 rpm

and 45°C. García et al. (2012) reported maximum hydrolysis yields of 60% for extraction of amino acids from 250 g/L of the freshwater strain *Scenedesmus almeriensis* using commercial enzymes Alcalase and Flavourzyme. Similarly, Zuorro et al. (2016) used different cocktails of enzymes to improve the lipids recovery from *Nannochloropsis* species. These authors reported an increase in lipids recovery from 16% to 140% compared to untreated biomass. To solve the problem of enzyme cost, both microbes excreting enzymes or immobilizing enzymes can be used. However, information about these two approaches is scarce (Yoo et al., 2014).

Oxidizing agents such as H_2O_2 or ozone react with cell wall constituents leading to degradation and subsequent disruption of the cell wall (Concas et al., 2015). This pretreatment can increase the extraction efficiency, but reaction times should be kept short to prevent oxidation of target compounds. Huang et al. (2016) applied excessive pressure-assisted ozonation and observed high disruption yield and high metabolite degradation. Ozonolysis proved to be attractive and promising with advantages over traditional methods, including low inhibitors' compounds production, minimal effect on carbohydrates, liquid phase absence, no chemical requirements, mild conditions, in-site and direct ozone production, and the generation of easily degradable subproducts. Drawbacks are high operational costs, toxicity, flammability, corrosivity, reactivity, and special materials for the equipment (Travaini et al., 2016). The selection of operation parameters must be well considered to minimize ozone consumption and generate by-products that might act as inhibitory compounds or impurities in downstream processes. The main process parameters are reactor design, moisture content, ozone concentration, ozone/air flow, and pretreatment time.

6.5 Combined methods for cell wall disruption

Pretreatment techniques can be combined to improve the efficiency of downstream processes. Freeze-drying is one of the most commonly used techniques prior to extraction of high-value products because of its mild operating conditions. Freeze-drying also enhances the efficiency of lipid extraction after milling (Halim et al., 2012b). Alternatively, the combination of ultrasonication or other disruption methods with different solvent systems to increase the efficiency and decrease the energy demand is interesting for the mild microalgae biorefinery (Passos et al., 2015b). Lorente et al. (2015) combined chemical treatment with steam explosion by applying acid steam explosion (120°C and 150°C; 5 min; 10%, w/w, H_2SO_4) for the extraction and release of lipids and sugars from three types of microalgae. After pretreatment at optimal conditions (10%, w/w, H_2SO_4, 150°C), the authors reported a near-complete sugar release and a twice higher lipid extraction based on extractable lipids. Likewise, Lee et al. (2014) applied hydrothermal nitric acid treatment from pure *Nannochloropsis* sp. and achieved 24.4% of lipid extraction at optimal conditions compared to 5.2% from untreated samples. Li et al. (2016) studied an oxidative chemical pretreatment (H_2O_2) with *Ulva prolifera* residue after hot water extraction to improve the enzymatic sugar hydrolysis yield. The authors reached 420 mg of reducing sugar release per gram of biomass compared to 232 mg/g for untreated biomass.

6.6 Recent developments

A more recently described mechanical disruption technology based on Henry's law is *explosive decompression*. An algae suspension is pressurized with gas and a sudden pressure drop expands the intracellularly dissolved gasses resulting in the formation of bubbles and rupture of cells. Some batch processes are described in literature with a variety of disruption agents in the gaseous or supercritical state (steam, CO_2, light alkanes, ammonia, etc.). For batch systems, often a long contact time is needed to allow sufficient diffusion of the disruption agent into the cells, and the technique is often limited to cells that are easily broken (Dierkes et al., 2009). For example, *pressurization with supercritical* CO_2 in patent US8148559 B1 requires a contact time of days (Walker et al., 2008). The time required for pretreatment can be improved significantly by using a continuous system such as that described in patent US2011/0183403 A1 (Dierkes et al., 2009). However, the latter process consumes high amounts of CO_2 (90 kg CO_2/kg suspension). Lower CO_2 amounts are described in patent EP2977439 (A1) (D'Hondt et al., 2014) in a continuous installation using liquid CO_2 as a disruption agent. The latter relies on the principle of efficient emulsification and mixing to increase the mass transfer between the phases. These technologies are promising for the valorization of sensitive compounds since they operate at low temperature, can handle high cell loadings, use no solvent, and have potential for upscaling.

Some mechanical techniques such as *laser*, *pulsed arc* (pulsed electronic discharge), and *atomic force microscopy* apply energy beams directly to cells (Günerken et al., 2015). This results in a very efficient energy transfer and thus low energy demand. Unfortunately, laser and atomic force microscopy are limited to lab scale or smaller and are thus more suited for fundamental research. Pulsed arc, on the other hand, is very aggressive because of high local temperatures. Another technique that focuses energy is high-frequency (3.2 MHz) focused ultrasonication, which proved to consume less energy than conventional ultrasonication (20 kHz), but efficient rupture would require the combination of both techniques (Wang et al., 2014).

Yoo et al. (2014) describe an *immobilized chemical disruption method* in which a surfactant immobilized on a membrane is used as a disruption agent. Cell disruption efficiency, however, was not so high (25.6%). Thermoresponsive polymers can obtain a disruption efficiency of about 32% and the combination of polymers with enzymes increased the disruption efficiency to 68% (Zheng et al., 2016). Alternatively, Hua et al. (2016) describe the use of a Ti_4O_7-based reactive electrochemical membrane for simultaneous harvesting and cell disruption to enhance lipid extraction.

Recent advances in *genetic engineering* of cyanobacteria (Gaj et al., 2013) and bacteriophages (Pál et al., 2014; Qazi et al., 2016) allowed the development of genome-editing strategies to perform precise and efficient disruption of cyanobacterial cells. Many of these strategies rely on the use of phage-encoded proteins that target the bacterial cell envelope and exhibit activity of peptidoglycan hydrolases (Gao et al., 2013). The insertion of bacteriophage lysis genes into the cyanobacteria genome controlled by inducible gene expression systems are among the most developed

approaches to date for bacteriophage-based cell lysis of cyanobacteria. A proof of a concept study using lysis systems derived from the bacteriophages infecting *Escherichia coli* and *Salmonella typhimurium* has demonstrated the feasibility of this cell wall disruption strategy in model cyanobacteria *Synechocystis* cultures, showing effective cell lysis mediated by nickel- or light-inducible promoters and release of the intracellular compounds into the liquid medium (Liu and Curtiss, 2009; Miyake et al., 2014). Possible limitations of this approach can be associated with growth rate reduction of genetically modified cyanobacterial strains or development of cell resistance to the specific chemical induction agents (Liu and Curtiss, 2009). Alternatively, phage-encoded peptidoglycan-degrading enzymes can be recombinantly expressed using, for example, *E. coli* or plant expression systems, and when applied exogenously to lyse cyanobacterial cells. However, due to the presence of an outer membrane in gram-negative bacteria, the exogenous application of bacteriophage-derived cell wall degrading enzymes is often used in parallel with outer membrane permeabilizing agents such as EDTA or organic acids (Oliveira et al., 2014; Lv et al., 2015). Such approaches require additional steps to be added in the cell disruption processes and may pose a risk of chemical contamination. This can be overcome using protein engineering enables the use of only the lytic domains of the lysins and combines them with various peptides (e.g., mediating the uptake through the outer membrane) to generate fusion proteins that are able to pass through the outer membrane and degrade peptidoglycan of gram-negative cells (Briers and Lavigne, 2015). Moreover, phage peptidoglycan hydrolases with intrinsic membrane-passaging capabilities has been recently identified in bacteriophages infecting the gram-negative bacteria *Acinetobacter baumannii* and some others (Lim et al., 2014). Therefore, although the exogenous application of peptidoglycan hydrolases for the disruption of cell wall of gram-negative organisms is in its early stages, this strategy holds strong promise for future applications. Other types of bacteriophage-based cell wall disruption strategies range from genetically modified bacteriophages with enhanced enzymatic activity enabling more effective degradation of extracellular polysaccharides and thus facilitating phage adsorption (Nobrega et al., 2015) to virion-associated peptidoglycan hydrolases (Rodríguez-Rubio et al., 2013) and construction of virus-like particles (VLPs) with lytic enzymes or other bactericidal agents either displayed on the surface of the VLPs or encapsidated within the VLPs (Westwater et al., 2003). The later approach was successfully applied to control population growth of the bloom forming dinoflagellate *Heterocapsa circularisquama* (Kang et al., 2015). In brief, genetic engineering as a tool may provide the alternative for conventional chemical and physical cell wall disruption methods although its economic feasibility for large-scale use still needs to be emphasized.

6.7 Cell disruption effectiveness and quality bioproducts from microalgae

The efficiency of extraction of intracellular metabolites from microalgae was found to differ according to the species, cell wall strength, and disruption method. To improve the extraction effectiveness, it is crucial to identify appropriate cell disruption

technologies and optimize the energy consumption. Mechanical disruption of cells is preferred in most cases as it proposes an approach that avoids chemical contamination of the bioproducts and preserves most of the functionality of intracellular material. They are common in large-scale processes due to their nonspecific disruptive nature and high efficiencies (Günerken et al., 2015). Furthermore, they are most effective and energy efficient at cell concentrations of 100–200 g/L (Greenwell et al., 2010), which makes them suitable to treat concentrated algae streams without the need of a drying step.

The most traditional mechanical microalgae pretreatments include bead milling, French press (high-pressure homogenization), ultrasonication, microwave techniques, and electric shock. The evaluated effectiveness of these treatments on different algal types in terms of disrupted cells, recovery of pigments, and proteins from processed biomass is summarized in Table 6.2. Bead milling has been widely used for extraction purposes and supports good cell disruption in many studies. High-speed and high-pressure homogenization is industrially well known for the extraction of algal products. Unfortunately, these treatments are less favorable for mild biorefining. Ultrasound and microwave-assisted extractions are now recognized as efficient extraction techniques that greatly reduce working times and increase yields and often the quality of the extract. The major disadvantage of ultrasonication is the relatively low cell disruption efficiency for some microalgae species. Meanwhile, microwaves are effective even for robust cell walls. Literature suggests that pulsed electric field treatment favors the extraction of lipids and proteins. However, this pretreatment is less suitable for fragile compounds.

Lorente et al. (2015) compared the effectiveness of different physical cell disruption approaches. Steam explosion of three types of microalgae biomass resulted in higher lipid extraction efficiencies compared to autoclaving, ultrasound, and microwave techniques.

Quality bioproducts refer to (functional) products at high concentration without contaminants or degradation products. In most of the published works, cell disruption effectiveness is evaluated by the yield of targeted products, but strong conditions reduce product quality. Downstream steps will then be more intensive, eventually decreasing overall yields and increasing process cost. Chemicals may influence the quality of the products due to the formation of by-products like inhibitors (e.g., furfural and 5-hydroxymethylfurfural). Moreover, a pretreatment applied to release one fraction can solubilize and degrade other biomass components, affecting the biorefinery concept (Yoo et al., 2014). Enzymes achieve high product quality but still remain expensive. In the case of ozonolysis, Schultz-Jensen et al. (2013) reported low inhibitor concentrations, less than 0.3 g/100 g dry biomass, without additional chemicals and with high disruption effectiveness in terms of ethanol yield. However, studies on ozonolysis on algal biomass are scarce. The quality of products released by ionic liquids and supercritical fluids is high compared to conventional pretreatments due to its self-separation and recyclability. However, the high prize of ionic liquids increases the process costs (Yoo et al., 2014). Moreover, Zhou et al. (2012) reported inhibitor compounds (furfural and 5-hydroximethylfurfural) in their work with ionic liquids since temperatures above 120°C caused carbohydrate degradation. Therefore,

Table 6.2 Comparison of different physical-mechanical techniques for microalga cell disruption effectiveness

Methods	Species	Outcome, product increase
Bead milling	*Botryococcus braunii, Chlorella* sp., *Chlorococcum* sp., *Scenedesmus quadricauda*	28.6%–99.9% disrupted cells
	Chlorella vulgaris, Synechocystis aquatilis	21%–38% (w/w) lipid yield
High-speed homogenization	*Haematococcus pluvialis*	Broken over 80% of cysts
	Nannochloropsis sp.	75.8%–78% of dry weight yield
High-pressure homogenization	*Chlorella* sp., *Chlorococcum* sp., *Nannochloropsis oculata*, *Nannochloropsis* sp., *Tetraselmis* sp.	50%–90% disrupted cells
	H. pluvialis cysts	2.2 mg/g DW, total carotenoids
	Nannochloropsis sp.	≈91% protein extraction
	Nannochloropsis sp.	8.5 times more oil extraction
Sonication	*Chlorococcum* sp.	4.5% disintegrated cells
	Botryococcus sp., *Chlorella protothecoides, C. vulgaris, Chlorella* sp., *Nannochloropsis* sp., *Scenedesmus dimorphus, Synechocystis aquatilis, Thalassiosira fluviatilis, T. pseudonana*	10.7%–52.5% (w/w) lipid yield
	Spirulina platensis	61.5% yield of β-carotene, ≈1.10 mg/g
	Scenedesmus obliquus	90% fatty acids and pigments
Microwave treatment	*Botryococcus* sp., *C. vulgaris, Scenedesmus obliquus, Scenedesmus* sp., *Nannochloropsis gaditana, Nannochloropsis* sp.	10%–77% (w/w) lipid yield
Pulsed electric field	*Synechocystis* sp.	Extraction similar to untreated cells
	Nannochloropsis salina	Fourfold more extraction with water than methanol extraction of untreated cells

Results included in the table are based on Huang et al. (2016), Al hattab and Ghaly (2015), Günerken et al. (2015), Joannes et al. (2015), Piasecka et al. (2014), Halim et al. (2012b), and Mendes-Pinto et al. (2001).

they emphasized on the need for operational parameter optimization to avoid biomass degradation and to include washing steps after the pretreatment.

The severity of thermal pretreatment is based on different factors, mainly particle size, temperature, pressure, and time. Severe conditions result in higher digestibility

but also in higher bioproduct degradation. Therefore, an increase of the effectiveness often results in a product quality decrease. Product quality is highly affected by degradation of algal compounds through cavitation during ultrasound pretreatment. This method is normally applied for sugar solubilization; nevertheless, some proteins get destroyed whereas lipids are hardly affected. Temperature control could reduce this degradation but also the cell disruption effectiveness. Microwave pretreatment provides higher process selectivity in terms of heating and the starter-stop times. The short process time as well as low inhibitor production is, however, counteracted by high operational costs. The feasibility at commercial scale is unrevealed, however, and studies for achieving scale-up would be necessary (Yoo et al., 2014).

Overall, the mildness of cell disruption directly affects product quality. Chemical and enzymatic treatments are highlighted as selective methods. Alternatively, the most promising techniques from the emerging technologies are continuous explosive decompression and immobilized (bio)chemical agents since they operate at low temperature, avoid solvents, and therefore facilitate downstream processing. Also contact with oxygen is limited, thus preventing oxidation of sensitive compounds. These factors should allow high quality, and if these emerging technologies improve, high disruption yields might be achieved as well.

6.8 Cell disruption in large scale

Currently bead milling, high-speed homogenization, and high-pressure homogenization are the main methods to disrupt algae in a large scale. Other established methods with potential for scale-up are ultrasound, microwave treatments, enzymatic lysis, chemical treatment, and pulsed electric field. However, low dry cell weight concentrations make ultrasound, enzymatic lysis, and pulsed electric field less interesting, and microwave effectiveness decreases with penetrating depth in batch processes.

6.9 Cost-effectiveness of disruption technologies

Cost-effectiveness in cell disruption is related to several factors such as energy consumption per kilogram of dry weight, dry cell weight concentration of the treated algae suspension, time to obtain reasonable disruption yields, consumables (biological, chemical, or mechanical disruption agents), product quality, required labor intensity, qualifications of personnel, equipment maintenance, CAPEX, and so on.

Several recent studies have discussed the cost-effectiveness of the previously mentioned physical-mechanical disruption technologies (Greenwell et al., 2010; Balasundaram et al., 2012; Coons et al., 2014; Al hattab and Ghaly, 2015; Artan et al., 2015; Günerken et al., 2015). Yet generalization is very complicated due to the high variety of methods and many unknowns. Primarily, cost effectiveness of disruption technologies depends on microalgae type, size, cell wall strength, growth stage, disruption method, process parameters, and the scale. Disruption of wet algal biomass is more preferred because it avoids expensive drying steps, but the cost also depends on the wet biomass concentration. Algae biomass concentrations

of 50–200 kg DW m^{-3} are considered as suitable for physical-mechanical disruption. Generally, bead milling, high-pressure homogenization, and microwave treatment are energetically among the most costly techniques whereas ultrasound and pulsed electric field are the cheapest (in investments and operational costs). Halim et al. (2013) calculated that the *actual energy needed for disruption* of single cell *Tetraselmis suecica* and *Chlorococcum* sp. using high-pressure homogenizer was similar for both species (5.9×10^{-5} and 6.4×10^{-5} J per cell) whereas a 20 times lower requirement was obtained with ultrasonication.

Specific energy consumption (SEC; kWh/kg dry biomass) was suggested by Günerken et al. (2015) as a process parameter for the comparison of energy requirements and cost-effectiveness. Energy consumption should be at least below the caloric value of algae, which varies from 4.2 to 6.4 kWh/kg dry biomass (Paine and Vadas, 1969). Energy requirements of physical-mechanical disruption methods vary widely (0.07–147 kWh/kg) whereas only microwave treatment fails to reach the target in all tests considered in Table 6.3. However, comparison based on SEC is only indicative and would need validation under similar pretreatment conditions. Doucha and Lívanský (2008) demonstrated that energy consumption could be reduced from 10.3 to 0.86 kWh/kg by altering bead milling parameters. Mechanical methods

Table 6.3 Comparison of the cost effectiveness of physical-mechanical cell wall disruption techniques

Disruption technique	Genus of microalgae	Power consumption, kWh/kg dry algal biomass
Microwave	*Botryococcus*, *Scenedesmus*, *Chlorella*, *Nannochloropsis*	17.3–116.7
High-pressure homogenizer	*Tetraselmis*, *Nannochloropsis*, *Chlorella*, *Chlorococcum*	0.25–146.9
Bead mill	*Chlorella*, *Botryococcus*, *Scenedesmus*	2.8–46.6
Ultrasound	*Chlamydomonas*, *Chlorococcum*, *Nannochloropsis*, *Tetraselmis, Spirulina*	1.6–36.7
Water bath	*Nannochloropsis*	4.7
Laser treatment	*Nannochloropsis*	3.7
Ball mill	*Chlorogloeopsis*	1.87
Heat exchanger	*Nannochloropsis*	2.3
Pulverizer	*Haematococcus*	3.5
High-speed homogenizer	*Nannochloropsis*	0.13
Pulsed electric field	*Isochrysis*	0.07

Power consumption was recalculated based on data from Artan et al. (2015), Balasundaram et al. (2012), Coons et al. (2014), and Günerken et al. (2015).

(e.g., bead mills) for cell disruption at industrial scale were optimized, but the energy demand was still high as only 1% of the energy was attributed to disruption. The remaining 99% of the applied energy was converted into heat and therefore required cooling. Balasundaram et al. (2012) innovated the bead milling process by altering the mechanism of energy transfer and reducing the energy consumption (1.87 kWh/kg) by 34% compared to the best algal disruption system reported.

Artan et al. (2015) suggested calculating energy requirements for various biofuel processes based on *fractional energy* (FE) defined as the ratio between the energy used in the extraction process and the total energy of the algal water content. The latter was the summation of lipid energy content and lipid-extracted algae. Based on their analysis, pressure homogenization to disrupt *Nannochloropsis oculata* exhibited the highest FE (1.86) followed by ultrasound technology (0.92) and a newly proposed heat exchanger system (0.33).

6.10 Conclusion

Cell disruption is crucial for the valorization of algal biomass and has already led to a variety of technology developments. Challenges remain to (1) disrupt all algae species of interest and (2) evolve further to scalable and economic attractive disruption approaches that preserve the cell constituents as much as possible.

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