Breakthroughs in bioalcohol production from microalgae: Solving the hurdles

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8.1 Introduction

The severe energy crisis during these last decades has made unavoidable the search and development of new renewable energy sources to help to make more sustainable and feasible processes and solve some environmental hurdles. As alternative to fossil fuels, different bioalcohols have been explored, such as butanol or ethanol. Bioethanol is the most studied bioalcohol due to its lower operational cost and higher yields (Voloshin et al., 2016). Bioethanol production has been widely studied and chronologically classified in different categories based on the origin of the feedstocks. First-generation bioethanol comes from food crops like sugarcane, sugar beet, corn, and wheat. However, ethical and economic issues are derived from using food crops and large agricultural lands for fuel production. Second-generation bioethanol have been used to try to solve these problems by replacing food crops with lignocellulosic materials, an overabundant raw material in the world (Alvira et al., 2010). Severe pretreatments are needed to break their strong structure, mainly due to their lignin content and the crystalline structure of cellulose (Lam and Lee, 2015). To the contrary, algae (the third-generation source) contain no lignin helping to overtake the drawbacks of the previous feedstocks, requiring only moderate pretreatments (Günerken et al., 2015). Advantages of microalgae biomass include their fast growth and productivity and the selective accumulation of lipids, proteins, or carbohydrates depending on the cultivation conditions. This chapter collects several studies related to different processes for bioalcohol production from microalgae biomass. In particular, topics like carbohydrate content of different algal biomass, methods to improve the accumulation of carbohydrates, pretreatments to disrupt the cell wall, enzymatic hydrolysis, and fermentation processes will be discussed.

8.2 Carbohydrate content of different algal biomass

In the microalgal cell, carbohydrates can be found in the outer cell wall (e.g., pectin, agar, alginate), the inner cell wall (e.g., cellulose, hemicellulose), and inside the cell as storage products (e.g., starch in microalgae and glycogen in cyanobacteria) as seen in Fig. 8.1.

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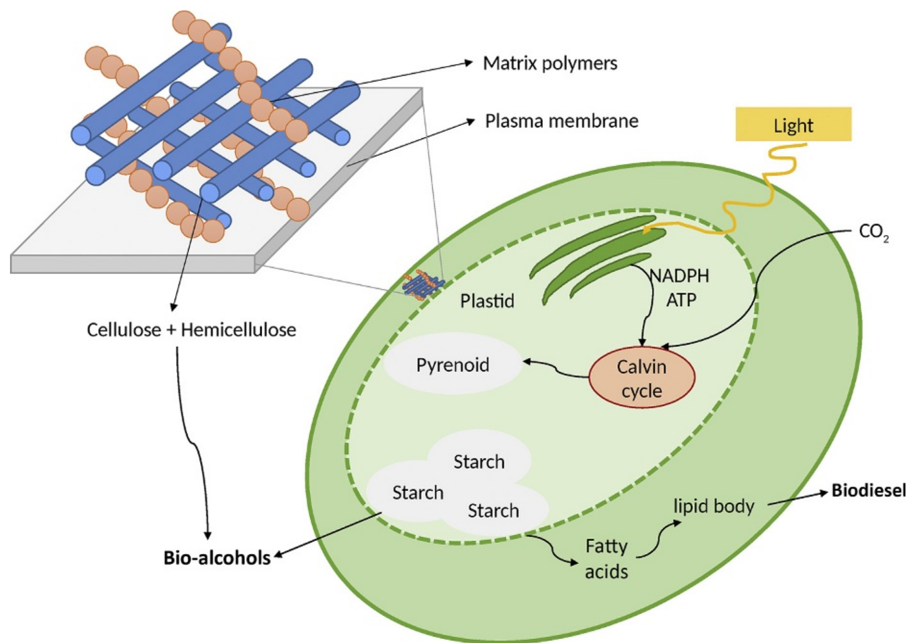


Fig. 8.1 Outline of carbon and energy storage routes in microalgae and their valorisation for biofuels production.

During photosynthesis, microalgae produce the monosaccharide glucose. This glucose is used as an energy and carbon source to produce proteins, lipids, and other carbohydrates. When irradiance is too high or when the inorganic nutrient supply is limited (e.g., nitrogen stress), the rate of glucose production during photosynthesis can exceed the rate of glucose consumption by the cell. This excess cannot be stored due to the disturbance of the cell's osmotic balance. Therefore, the overproduced glucose is converted either into polysaccharides or into lipids, which will act as carbon and energy storage for future use. Because glucose conversion into polysaccharides is much faster than into lipids, microalgae will often first accumulate carbohydrates and afterward lipids (Ho et al., 2012).

Some photosynthetic microorganisms, such as many cyanobacteria, accumulate carbohydrates only as an energy and carbon reserve. Cyanobacteria store glucose in form of glycogen, an α-(1-4) polymer with many α-(1-6) glucan branches. This glycogen forms more or less soluble globules of around 55,000 glucose units with a diameter of about 42 nm (Ball et al., 2011). Starch is the glucose storage form of green and red algae. It consists of a very large, insoluble α-(1-4) polymer of 10^5–10^6 glucose units with less α-(1-6) branches than glycogen. The glucose polymers form helices that align and form a semicrystalline structure. Euglenophytes and diatoms store glucose in the form of β-(1-3), β-(1-6) glucans (paramylon or laminarin).

Carbohydrates can also be found in the microalgal cell wall. Most microalgal cell walls contain cellulose (β-(1-4) glucan). Multiple cellulose chains are linked by hydrogen bonds to form a complex and crystalline structure that is resistant to enzymatic

degradation (Popper and Tuohy, 2010). Most microalgae also contain hemicellulose, a polysaccharide composed of different types of monosaccharides (as mannose, xylose, galactose, rhamnose, and arabinose) connected by β-(1-4) and occasionally β-(1-3) glycosidic bonds (Cheng et al., 2015). In addition, microalgae cell walls also contain matrix polysaccharides. These include sulfated polysaccharides similar to agar or carrageenan (in red algae) (Popper et al., 2011), polysaccharides containing carboxylated monosaccharides (uronic acids) such as alginate (in brown algae) (Templeton et al., 2012) and amino sugars (Ortiz-Tena et al., 2016). Cyanobacterial cell walls also contain peptidoglycan, which is a cross-linked heteropolymer formed by β-(1-4)-linked *N*-acetylglucoseamine and *N*-acetyl-muramic acid (Hoiczyk and Hansel, 2000). Microalgae cell walls may display a high degree of complexity with crystalline as well as amorphous or gelatinous layers and containing nonpolysaccharide polymers such as algaenan (cutinlike compound). This complexity makes microalgae cell walls quite recalcitrant. Besides, microalgae can also excrete them to the medium. These exopolymers released may represent up to 17% of total photosynthesis (Hulatt and Thomas, 2010). Excreted sugar concentrations strongly differ between species and can vary from 10 to 100 mg L^{-1} (Myklestad, 1995). Some may be loosely bound to the cell whereas others are freely dissolved in the medium. These exopolysaccharides are often complex polymers containing many different types of monosaccharides, some of which are modified by methyl, acetyl, sulfate, carboxylic acid, or acetylamine groups (Delattre et al., 2016).

8.3 Methods for increasing carbohydrate content of the algal biomass

When microalgae are cultivated under unrestricted and favorable environmental conditions, carbohydrate content is typically around 10%–30% (see Table 8.1). Despite the relative low carbohydrate content, microalgae composition could be altered to cope with the stress provoked by unfavorable environmental conditions. In most cases, stress conditions do not have to be necessarily detrimental for microalgae to synthesize and accumulate carbonaceous compounds (lipids or carbohydrates). The carbohydrate accumulation could be an interesting option in the field of bioethanol production, although in most of the cases, stress conditions hinder biomass growth (see Section 8.3.3). Therefore, it is very important to find and optimize strategies and methods for the manipulation of environmental factors having an effect on carbohydrate accumulation (Markou et al., 2012). Several studies have suggested the use of metabolic engineering for the development of carbohydrate accumulating strains (Radakovits et al., 2010), but in this chapter, only biochemical engineering (i.e., strategies and methods related to environmental (cultivation)) conditions will be discussed. Nutrient availability, light intensity, temperature, and pH are the most influential factors on the microalgae biomass composition.

8.3.1 Nutrient availability

Besides light and CO_2, various elements, such as nitrogen, phosphorus, potassium, and sulfur are required for microalgae cell growth. Their availability affects microalgae growth since nutrient requirements and ratio depend on the microalgae species. Moreover, the limitation of a particular nutrient could have a significant impact on the

Table 8.1 Carbohydrates present in different microalgae species (Lam and Lee, 2015; Suganya et al., 2016)

Microalgae species	Total carbohydrate content (% dry mass)
Chlamydomonas reindhardtii	17
Chlorella pyrenoidosa	26
Chlorella sp.	19
Chlorella vulgaris	12–17
Chlorococcum sp.	32.5
Dunaliella bioculata	4
Dunaliella salina	32
Euglena gracilis	14–18
Isochrysis galbana	7.7–13.6
Isochrysis sp.	5.2–16.4
Mychonastes afer	28.4
Nannochloropsis oculata	8
Porphyridium cruentum	40
Prymnesium parvum	25–33
Scenedesmus abundans	41
Scenedesmus dimorphus	21–52
Scenedesmus obliquus	15–51.8
Spirogyra sp.	33-64
Spirulina platensis	8–20
Spirulina maxima	13–16
Synechoccus sp.	15
Tetraselmis maculate	15
Tetraselmis sp.	24
Tetraselmis suecica	15–50

biochemical composition (Kamalanathan et al., 2015). The most important effects are pigment degradation (chlorophyll and phycocyanin, which are proteins) and accumulation of either lipids or carbohydrates. Even though a close relation between the metabolic pathways and lipid or carbohydrate biosynthesis has been demonstrated, the accumulated macromolecule will depend on the microalgae species, the stress conditions encountered during cultivation, and the growth stage (Fernandes et al., 2013). For this reason, there are frequently contradictory results reported in the literature concerning lipid or carbohydrate accumulation.

8.3.1.1 Nitrogen

Nitrogen is the second most abundant element in microalgae biomass. It is required to synthetize various essential biomolecules (proteins, DNA, and pigments). Microalgae can utilize different forms of nitrogen (nitrate, ammonium/ammonia, urea, and organic nitrogen such as amino acids). Some differences in the biochemical composition of microalgae biomass grown with different nitrogen forms have been reported (González-Fernández and Ballesteros, 2012). Nevertheless the rate of carbohydrate accumulation obtained using different forms of nitrogen is rather low compared to the nitrogen limitation method. Abundant published studies investigated the effect of nitrogen supply

on biochemical composition. Most of them are dealing with lipid accumulation (Benvenuti et al., 2015; Negi et al., 2016) and fewer on carbohydrate accumulation (Depraetere et al., 2015a,b). Nitrogen starvation changes the flow of the photosynthetically fixed carbon from the metabolic pathway of protein synthesis to the lipid or carbohydrate, resulting in their accumulation. However, there are differences among species; whereas oleaginous eukaryotic microalgae tend to store energy in the form of lipids, the rest of algae and cyanobacteria tend to produce carbohydrates. Key enzymes that are affected by nitrogen limitation include carbonic anhydrase, ribulose-1,5-bisphosphate carboxylase/oxygenase, starch synthase/glycogen synthase, sucrose synthase, and sucrose phosphate synthase (González-Fernández and Ballesteros, 2012). Under nitrogen starvation conditions, *Chlorella vulgaris* displayed an accumulation of carbohydrates up to 38%–41% (Brányiková et al., 2011) and *Tetraselmis subcordiformis* about 35% (Ji et al., 2011) whereas the cyanobacteria *Spirulina maxima* displayed around 60%–70% (De Philippis et al., 1992) and *Spirulina platensis* about 55%–65% (Sassano et al., 2010).

8.3.1.2 Phosphorus

Phosphorus plays a key role on the vital biomolecule synthesis and participates on essential metabolic processes. When phosphorus is limited, microalgae and cyanobacteria tend to accumulate carbohydrates (Brányiková et al., 2011; Markou et al., 2012) even though lipid accumulation under phosphorus limitation is also reported (Challagulla et al., 2015).

Carbohydrate synthesis is not a phosphorus-consuming process. Since the controlling enzyme for the synthesis of carbohydrates (ADP-glucose pyrophosphorylase) is activated by the 3-phosphoglycerate enzyme, it is inhibited by the presence of inorganic phosphorus and the degree of carbohydrate accumulation is determined by the ratio of 3-phosphoglycerate to inorganic phosphorus (Gómez-Casati et al., 2003). Microalgae biomass accumulates carbohydrates when the intracellular phosphorus drops below a threshold limitation level (Cade-Menun and Paytan, 2010). In the cyanobacterium *S. platensis*, carbohydrates started to accumulate when the intracellular phosphorous was lower than about 4 mgP $g_{algae\ dw^{-1}}$ (Markou, 2012). Similarly, the highest biomass carbohydrate content is reached when the intracellular phosphorus concentration drops to its lowest possible level. For *S. platensis*, the typical intracellular phosphorus content is around 0.8%–1% but it can be reduced to 0.2% or even lower, which means that cell numbers can be multiplied 3.5 to 5 times at the expense of the intracellular phosphorus before cells reach the minimum intracellular concentration (Markou, 2012). Phosphorus limitation has a significant effect on carbohydrate accumulation; for example, phosphorus-limited *Chlorella* sp. accumulated carbohydrates up to 55% (Brányiková et al., 2011) and *S. platensis* up to 63% (Markou et al., 2012) from an initial content of about 10%–20%.

8.3.1.3 Other nutrients

Likewise, microalgae cultures grown under the limitation of other nutrients like sulfur, potassium, and manganese also display an accumulation of carbohydrates. In sulfur-limited cultures of *Chlamydomonas reindhardtii*, Melis (2007) reported a

10-fold carbohydrate content increase, and Ball et al. (1990) 10, 15.5, and 4.5 increase in carbohydrates in autotrophic, mixotrophic, and heterotrophic cultures, respectively. In addition, manganese and potassium starvation in cultures of the same species resulted in the increase of carbohydrate content (Ball et al., 2011). Brányiková et al. (2011) suggested that sulfur limitation is the most suitable strategy for the production of carbohydrate-rich microalgae because cells maintain a high carbohydrate content (about 60%) for longer time compared to other nutrient starvation methods (nitrogen and phosphorus) before cells enter the cell-death phase. Since the production of biohydrogen consumes carbohydrates, sulfur, or potassium, limitations have been suggested also as potential strategies employed for biohydrogen production using microalgae (Torzillo et al., 2014).

8.3.2 Other factors

Besides nutrient limitation, which may be the most effective way to trigger carbohydrate (or lipid) accumulation, stress conditions associated with other environmental/operational cultivation parameters have been proposed for the manipulation of biomass biochemical composition and hence carbohydrate accumulation. Parameters that could be used to accumulate carbohydrates are high light intensity and high salinity (Brányiková et al., 2011; Markou and Nerantzis, 2013).

Light is commonly used by microalgae/cyanobacteria to fix carbon through photosynthesis. The quality and the quantity of light affect biomass growth rates and also influence biomass composition (Markou, 2014; Khajepour et al., 2015). Biomass growth rates increase as the light intensity increases up to a maximum level (typical saturation intensity is 200–400 $\mu mol_{photons}\ m^{-2}\ s^{-1}$) whereas a further increase may inhibit photosynthesis (Lu and Vonshak, 1999). In general, high light intensities result to an increase in the carbohydrates (Hu, 2003); for example, the cyanobacteria *Porphyridium* and *Arthrospira* displayed a 300% and 34% increase in carbohydrate content, respectively, when light intensity increased (De Philippis et al., 1992; Aikawa et al., 2012).

Under high salinity, microalgae typically respond by accumulating intracellular carbohydrates of low molecular weight to adjust the intracellular pressure and protect themselves from osmotic lysis (Rao et al., 2007). Accordingly, carbohydrate content was increased to 35.91% under 400 mM NaCl stress in *Scenedesmus* sp. microalgae cultures (Pancha et al., 2016). The manipulation of salinity along with nutrient limitation has been proposed as an effective strategy for carbohydrate accumulation (Yao et al., 2013).

8.3.3 Effect of stress on growth rates

In most cases, triggering carbohydrate accumulation by a stress factor results in decreasing growth rates and therefore low biomass productivities. This is mainly caused by physiological and morphological alterations caused by the stress conditions that hinder cell division or even cause cell lysis and death. For this reason, optimization of the cultivation process is required for overcoming this negative effect. Stress

factors should be adjusted at levels that may allow the highest biomass production along with the highest carbohydrate accumulation possible or although the biomass production is low, the production of carbohydrates must be enough to make bioethanol production feasible. A process optimization regarding the nutrient limitation has been suggested by considering the minimum intracellular nutrient concentration (i.e., by supplying the appropriate amount of nutrients to avoid a decrease on biomass production) while triggering as much as possible carbohydrate accumulation (Markou et al., 2012). An alternative strategy is to develop a two-stage culture system in which biomass would be produced under optimum conditions at a first stage and then the cells would be exposed to stress conditions at a second stage, consequently altering their composition (Rodolfi et al., 2009).

8.4 Pretreatments: Effect on release of sugars and degradation compounds

Different pretreatments could be applied to disrupt the cell wall, liberate the polysaccharides, and hydrolyze them to simple sugars. The efficiency of these processes highly depends on biomass type and composition. Thus, the optimal disruption method should be chosen for maximizing fermentable sugar yields while minimizing product degradation and operating costs. The most common methods for microalgae biomass treatment devoted to sugar release are described in the following sections.

8.4.1 Physical-mechanical methods

Physical-mechanical pretreatments entail the physical modification of biomass owing to pressure, low to moderate temperature, or shear forces. In general, they are considered more effective than other types of pretreatments, showing low formation of degradation products. However, they often require higher energy input, and they are not specific, making no distinction among different biomass fractions. Proteins, lipids, and carbohydrates are equally liberated, which may reduce the economic feasibility and decrease the quality of derived bioproducts.

Milling or thermal methods have been usually applied for lipid extraction and biogas production enhancement (Passos et al., 2015). Bead milling is a high-efficiency disruption method. Biomass is subjected to mechanical forces and shear stress by the contact with beads in movement, breaking down the recalcitrant cell walls in an easy way. Its main parameters are bead type (loading, size, and material), feed rate, biomass properties (species and humidity), and time. Few references could be found regarding their application on bioethanol production. Günerken et al. (2016), using the bead milling process (3% w/w biomass concentration, 45 min, 0.4–0.6 mm zirconia beads, 2000 rpm) for both ND (N-depleted) and NR (N-repleted) *Neochloris oleoabundans* cultures (initial carbohydrates of 17% and 14.4%, respectively), found 0.12 and 0.05 $g_{sugar}g_{algae\ dw^{-1}}$ of released sugars in the supernatant, accounting for 68% and 34% sugar release yield, respectively. Miranda et al. (2012) applied the same pretreatment (5 cycles of 1 min

followed by 1 min in an ice bath) to an aqueous suspension of *Scenedesmus obliquus* biomass (31.8% of initial carbohydrates) and obtained a sugar release yield lower than 0.03 $g_{eq\ glucose} g_{algae\ dw^{-1}}$. This discrepancy in the results could be attributable to the different pretreatment conditions as well as different cell wall conformation among species.

Thermal pretreatments have classically been applied to enhance the breakdown of particulate organic matter in anaerobic digestion using temperatures from 50°C to 270°C whereas, for bioethanol production, this temperature varies from 55°C to 170°C. Nevertheless, the generation of recalcitrant compounds associated with high temperatures could contribute to reducing bioethanol yields. Pretreatments applying temperatures over 120°C are considered in this chapter as combined pretreatments by the combined pressure and temperature effects. For example, Miranda et al. (2012) also tested thermal pretreatment (120°C and 1.2 bar for 30 min) in their previously cited work, obtaining with this method 0.04 $g_{eq\ glucose} g_{algae\ dw^{-1}}$, just 12.6% of total sugar-release efficiency.

On the other hand, even though freezing and freeze-drying methods are not considered as a pretreatment, certain authors have reported the effect of low-temperature storage methods when comparing the efficiency of pretreatments on biogas production from fresh, frozen, and freeze-dried microalgae biomass (Gruber-brunhumer et al., 2015).

8.4.2 Chemical methods

The use of chemicals has been widely reported by many researchers such as Harun and Danquah (2011) and Ho et al. (2013). Different reagents will have diverse effects on biomass depending on the microalgae species, its carbohydrate composition, and the nature of the selected chemical as seen in Table 8.2. Acids usually manage to open the cell wall membrane whereas alkali tends to saponify membrane lipids. Their main parameters are the type of chemical and its concentration, temperature, time, biomass concentration, and microalgae species (Lam and Lee, 2015).

Acid and alkali are the classic chemicals tested for bioethanol production. These pretreatments are fast and relatively inexpensive with acids providing higher sugar yields (up to 100%) than alkali (Harun et al., 2011). Concentrated reagents could be apparently considered more efficient, but they have some drawbacks such as generation of degradation compounds, equipment corrosion, and high operational and preservation costs. They also require a pH readjustment prior to the subsequent fermentation step in accordance with the optimal of the fermentative microorganism. Other main process variables are temperature and time, usually applied inversely: high temperatures (>100°C) with short times (<15 min), or low temperatures (<100°C) with longer times (30–90 min).

Regarding acid methods, H_2SO_4 is the most applied acid although HCl, H_3PO_4, and HNO_3 are also used. Nguyen et al. (2009) studied the influence of concentration (1%–5% v/v H_2SO_4), temperature (100°C, 110°C, and 120°C) and time (15–120 min) when applying acid hydrolysis for sugar extraction from *C. reindhardtii* UTEX 90 biomass

Table 8.2 **Sugar release from different microalgae biomasses via chemical pretreatment methods**

Microalgae species	Method	Conditions	Total carbohydrates (%)	Sugar release yield (g/g algae)	References
Chlorella vulgaris	Acid	1% (v/v) H_2SO_4, 121°C, 120 min	50.4	0.472	Ho et al. (2013)
Scenedesmus bijugatus (Postlipid extraction)	Acid	2% (v/v) H_2SO_4, 130°C, 45 min	26.0	0.218	Ashokkumar et al. (2015)
Chlamydomonas reindhardtii	Acid	3% (v/v) H_2SO_4, 110°C, 30 min	60.0	0.580	Nguyen et al. (2009)
Scenedesmus obliquus	Acid	2 N H_2SO_4, 120°C, 30 min	31.8	0.286	Miranda et al. (2012)
Spirulina platensis	Acid	0.5 N HNO_3, 100°C, 180 min	58.0	0.522	Markou et al. (2013)
Scenedesmus obliquus	Alkali	3 N NaOH, 120°C, 30 min	31.8	0.025	Miranda et al. (2012)
Chlorella sp.	Ionic liquid	[Emim][Cl]+HCl 7 wt%, 105°C, 3 h	73.6	0.648	Zhou et al. (2012)
Chlorella sp.	Ionic liquid	[Emim][Br]+HCl 7 wt%, 105°C, 3 h	73.6	0.564	Zhou et al. (2012)
Chlorella sp.	Ionic liquid	$[N_{2,2,2,2}]$[Cl]+HCl 7 wt%, 105°C, 3 h	73.6	0.483	Zhou et al. (2012)
Scenedesmus obliquus biomass from wastewater treatment	Alkaline peroxide	H_2O_2 7.5% (w/w), 50°C, 1 h	15.7	0.09	Martín Juárez et al. (2016)

with 60% (w/w) of initial carbohydrates (35% of starch). They selected as optimal conditions 3% H_2SO_4, 110°C, and 30 min, achieving a glucose release of 0.58 $g_{glucose}\ g_{algae\ dw^{-1}}$. Other types of acids were tested by Markou et al. (2013), who applied H_2SO_4, HNO_3, HCl, and H_3PO_4 at four concentrations (2.5, 1, 0.5, and 0.25 N) and four temperatures (40°C, 60°C, 80°C, and 100°C) to pretreat *S. platensis* (58% initial carbohydrates). This study showed that best conditions (0.5 N HNO_3, 100°C, and 180 min) resulted in a sugar yield of 0.522 $g_{sugar}g_{algae\ dw^{-1}}$, which corresponds to an efficiency of 90%.

For alkali methods, NaOH is the most studied reagent. Harun et al. (2011) observed a 64.74% reduction on average particle size and obtained a sugar yield of 0.350 $g_{glucose}g_{dw^{-1}}$ from alkali-treated samples (0.75% w/v NaOH at 120°C for 30 min) of *Chlorococcum infusionum* biomass (initial carbohydrate content of 32.52%), attributed to the cleavage of intermolecular linkages between hemicellulose and other polymeric components on the cell wall. Nevertheless, Miranda et al. (2012) reported a yield of just 0.025 $g_{eq\ glucose}g_{algae\ dw^{-1}}$ after NaOH pretreatment (NaOH 3 N, 120°C, 1.2 bar, 30 min) of *S. obliquus* (31.8% carbohydrate content) when 0.082 $g_{eq\ glucose}g_{algae\ dw^{-1}}$ were released by acid hydrolysis with H_2SO_4 at identical conditions. These values correspond to an efficiency of 7.9% and 25.8%, respectively. The authors assumed that the high alkali concentration used caused severe sugar degradation, but no analysis was performed to corroborate this fact.

Other methods like ozonolysis, alkaline-peroxide, and ionic liquids are emerging to soften the harsh conditions that are generally required by acid/alkali. Ozonolysis has been applied for lignocellulosic biomass delignification and macroalgae pretreatment. It involves the reaction between ozone and the cell wall protective components (lignin for lignocellulosic materials or carbohydrates for algae). Some advantages are the absence of a liquid phase, mild conditions, and on-site ozone production. However, it has high toxicity, flammability, corrosivity, and reactivity; hence special materials for the equipment are required, increasing its costs (Travaini et al., 2016). The main process parameters are reactor design, moisture, ozone concentration, ozone/air flow rate, and time. Schultz-Jensen et al. (2013) reported low degradation compound formation from ozonated *Chaetomorpha linum* with complete glucan and arabinan recovery in the solid fraction and 75% xylan recovery. Alkaline-peroxide method uses H_2O_2 and NaOH as chemicals, providing high glucose yield working at moderate temperatures when applied on lignocellulosic biomass (Rabelo et al., 2014). Martín Juárez et al. (2016) applied this pretreatment (1 h, 50°C, and H_2O_2 concentrations from 1% to 7.5% w/w) to different microalgae biomass cultivated in domestic and pig manure wastewaters, obtaining the best sugar yield (0.06 $g_{sugar}g_{algae\ dw^{-1}}$, which corresponds to a 38.3% solubilized sugar recovery beside a certain degradation) for freeze-dried *S. obliquus* biomass grown in domestic wastewater and pretreated with a concentration of 7.5% (w/w) H_2O_2.

Other options are ionic liquids. Their exceptional properties (low volatility, high dissolving power, ease and completeness of recovery from water, great variety) make them a promising method for disrupting and degrading algal biomass, but their application to large-scale processes is still a challenge due to their elevated

cost (Yoo et al., 2014). Ionic liquids have been quite widely explored for lipid extraction for microalgae but is still a relatively new method for bioethanol production. For instance, Zhou et al. (2012) obtained 0.65 $g_{sugar}g_{algae\ dw^{-1}}$ applying [Emim]Cl and 7% (w/w) HCl at 105°C for 3 h to *Chlorella* sp. (73.58% of initial carbohydrates).

8.4.3 Combined pretreatments

In this section, pretreatments that cannot be classified in previous sections are addressed since they involve different chemical and/or physical effects: hydrothermal pretreatment, steam explosion, ultrasonication, microwave use, HPH, supercritical fluids, and diverse combinations of the different methods explained before (Table 8.3).

High-temperature pretreatments consist on biomass degradation at temperatures higher than 120°C, usually associated with high pressure. Depending on how the pressure is relieved, they can be classified as hydrothermal pretreatments and steam explosion. The term *hydrothermal* includes several processes where water suffers changes in its physicochemical characteristics because of temperature and pressure augmentation, and it is a characterized by a smooth pressure relief once reaction time has finished. These changes have a deep impact on the reaction outcome, leading to a great variety of products. Since biomass fractions (including probable released sugars) suffer chemical conversion in other different compounds due to harsh conditions, these processes are scarcely considered for bioethanol production (Carrere et al., 2016). Nevertheless, Mendez et al. (2014) studied the effect of this pretreatment (140°C, 160°C, and 180°C; 3, 6, and 10 bars; 10 and 20 min) on the solubilization of different fractions of *C. vulgaris* (36.6% of carbohydrates), achieving 69% of carbohydrate solubilizations at 180°C, 10 bar, and 10 min. In steam explosion, saturated steam is applied at a high temperature (140–170°C) at the corresponding pressure (3.6–7.9 bar) for several minutes (5–30 min), but in contrast to hydrothermal processes, pressure is afterward suddenly released and biomass is quickly transported to a flash vessel and cooled. This instantaneous pressure drop leads to cell wall rupture and biomass disintegration. This process is commonly applied on lignocellulosic biomass for bioethanol production, but in the case of microalgae, this pretreatment has been investigated only in batch tests for biogas production enhancement. Nonetheless, Lorente et al. (2015) applied steam explosion to *Nannochloropsis gaditana* (initial carbohydrates of 13.5% dry ash-free basis) at 120°C and 150°C held for 5 min. Both temperatures led to a 0.06 $g_{sugar}g_{algae\ dw^{-1}}$ (44.4% of sugar solubilization yield).

Ultrasonication consists of high-frequency acoustic waves going through a medium. These waves stretch the molecular spacing of this medium, forming microbubbles due to sudden expansion and producing violent collapses, thus generating chemical and mechanical effects (Kim et al., 2016). This cavitation promotes microalgae cell wall breakdown and organic matter solubilization. Controllable parameters are mainly output power and exposure time, which define the specific energy (Passos et al., 2015). However, temperature, microalgae species and biomass concentration will also influence the results. For example, Jeon et al. (2013) observed a carbohydrate solubilization of 32.4% when subjecting *S. obliquus* (initial content of 37%) to

Table 8.3 **Sugar release from different microalgae biomass via combined pretreatment methods and new techniques**

Microalgae species	Method	Conditions	Total carbohydrates (%)	Sugar release yield (g/g algae)	References
Chlorella vulgaris	Hydrothermal	180°C, 10 min	36.3	0.250	Mendez et al. (2014)
Scenedesmus obliquus	Ultrasound	2200 W, 15 min	–	0.450	Choi et al. (2011)
Scenedesmus obliquus	Ultrasound	2200 W, 15 min	37.0	0.120	Jeon et al. (2013)
Chlorella sp.	Ultrasound	800 W, 80 min, 1.52 L min^{-1}	–	0.370 (glucose)	Zhao et al. (2013)
Scenedesmus obliquus	Ultrasound	200 W, 30 s, 5 cycles	31.8	0.020	Miranda et al. (2012)
Nannochloropsis spp.	Ultrasound	200 W, 600 s, pH 8.5	30.0	0.030	Parniakov et al. (2015)
Chlorella sorokiniana	Microwave	150 W, 40 s	–	0.021	Hernández et al. (2015)
Scenedesmus obliquus	High pressure homogenization	24,000 rpm, 5 min, 2 cycles	31.8	0.031	Miranda et al. (2012)
Lipid-extracted *Chlorococum* sp.	Supercritical fluids	CO_2, 60°C, 400 mL min^{-1}	43.8	0.358	Harun et al. (2010)
Nannochloropsis spp.	Pulsed electric field	20 kV/cm, 30°C, 4 ms, pH 8.5	30.0	0.027	Parniakov et al. (2015)

ultrasonication for 15 min, liberating 0.12 $g_{sugar}g_{algae\ dw^{-1}}$. This solubilization caused a reduction in suspension hydrophobicity from 75% (untreated) to 54% (15 min) due to the hydrophilic nature of carbohydrates released by cell lysis. Recently, Parniakov et al. (2015) pretreated *Nannochloropsis* spp. (30% of carbohydrates) and investigated the effect of pH on extraction efficiency. They obtained 0.030 $g_{eq\ glucose}g_{algae\ dw^{-1}}$ applying 200 W, 600 s, and pH 8.5, which means 10% solubilization efficiency.

Microwave offers another method with effects similar to those of ultrasound. When an aqueous suspension is exposed to short electromagnetic waves, they selectively excite the polar water molecules, causing local heating and hence pressure increase. These effects combined with the microwave-induced cell membrane damage facilitate the release of intracellular metabolites (Günerken et al., 2015). Its main advantages are high effectiveness, robustness, and easy scale-up thanks to its simplicity (Lee et al., 2010). Additionally, temperature increase is more homogeneous, reducing heat-related degradation. The main parameters that can be controlled are output power and exposure time. This technology has mainly been studied for lipid extraction and enhancement of biogas production (Passos et al., 2015). Hernández et al. (2015) applied microwave treatment for carbohydrate release from *Chlorella* sp. (18% of initial carbohydrate content). This study obtained a sugar yield up to 0.021 $g_{sugar}g_{algae\ dw^{-1}}$ at 150 W for 40 s.

Other method taking advantage of cavitation is homogenization. There are two types: high-pressure homogenization (HPH or French press) and high-speed homogenization (HSH). The sudden pressure drop, caused by a high stirring speed (HSH) or by impact of pressurized (around 150 MPa) biomass (HPH), provokes hydrodynamic cavitation. The applied pressure is the main operable parameter even though temperature, number of passes, setup design, and flow rate may be also decisive (Lee et al., 2012). Its main advantages are low heat formation and thus scarce thermal degradation, no dead volume in the reactor, and easy scale-up, but it is a highly energy-demanding process. This is a well-known method for microbial product extraction and sterilization (Kim et al., 2013), but few references can be found for microalgae biomass. Miranda et al. (2012) tested HSH (2 cycles at 24,000 rpm for 2.5 min) but obtained little effect on cell disruption of *S. obliquus* (31.8% of initial carbohydrate content) with a sugar release lower than 0.03 $g_{eq\ glucose}g_{algae\ dw^{-1}}$ and hence a sugar solubilization yield below 10%.

Supercritical fluid application is a relatively new technique whose principal advantage is the combination of properties of liquid and vapor states. CO_2, ethane, methanol, ethanol, benzene, and water are currently being explored as suitable supercritical fluids (Sawangkeaw et al., 2010). CO_2 is one of the most vigorously investigated supercritical fluids for pharmaceutical and other health-related product extraction from microalgae (Jaime et al., 2007), and its use for sugar extraction seems to be a possible alternative. CO_2 is nontoxic, inexpensive, highly miscible, and abundantly available with mild critical conditions (31°C and 1072 psi), which allow most compounds to be easily and safely extracted. Nonetheless, high power consumption and difficult scale-up are challenges still to be solved (Mohan et al., 2013). For example, Harun et al. (2010) studied the fermentation suitability of *Chlorococum* sp. biomass

(43.8% of initial carbohydrate content) after supercritical CO_2 lipid extraction (60°C, 400 mL min^{-1}). They reported a 60% increase on ethanol production after lipid extraction compared to intact microalgae biomass. During the supercritical fluid extraction of lipids, the cell wall of the microalgae is ruptured due to the high temperature and pressure required for the process, releasing cell wall carbohydrates.

Different types of the above mentioned pretreatments can be combined, usually for reducing the energy requirements of physical pretreatments or the reagent quantities needed on chemical pretreatments. Lorente et al. (2015) applied an acid steam explosion (120°C and 150°C, 2 and 4.7 bar; 5 min; 10%, w/w, H_2SO_4) for the sugar release from single cultures of *N. gaditana*, *Chlorella sorokiniana*, and *Phaeodactylum tricornutum* with 13.5%, 40.3%, and 13.2% dry ash-free basis of carbohydrates, respectively. After the pretreatment at optimal conditions (150°C, 4.7 bar), sugar release achieved values of 0.117 $g_{sugar}g_{algae\ dw^{-1}}$, 0.382 $g_{sugar}g_{algae\ dw^{-1}}$, and 0.127 $g_{sugar}g_{algae\ dw^{-1}}$, respectively, for each microalgae biomass that corresponded to sugar yields of 87%, 95%, and 96%.

Finally, new techniques for microalgae cell disruption are rapidly emerging, including explosive decompression, laser treatment (McMillan et al., 2013), microfluidizer, pulsed arc (Boussetta et al., 2013), high-frequency-focused ultrasonication (Wang et al., 2014), and cationic polymer-coated membranes (Yoo et al., 2014), which could be useful in future on an industrial scale for bioalcohol production processes.

8.5 Hydrolysis: Sugar recovery from microalgae

Some authors contemplate enzymatic hydrolysis as another pretreatment. However, in this chapter, this saccharification process by means of enzymes is classified as a separate and additional step that can be applied after some of the pretreatments explained in Section 8.4 lessening their severity or alone and hence acting as a pretreatment itself.

Enzymatic hydrolysis should be tailor made depending on the type of carbohydrate to be hydrolyzed (cellulose, hemicellulose, glycogen, and/or starch). For cellulose, its β-(1–4)-glucosidic linkages on the amorphous areas are randomly hydrolyzed by endo β-(1–4)-glucanase, creating new chain ends. The exo β-(1–4)-glucanase acts at the nonreducing ends of the cellulose molecule and cellodextrins, liberating cello-oligomers and cellobiose units (each unit containing 2 β-(1–4)-bonded glucose molecules). The last step is the hydrolysis of these β-linkages of cellobiose molecules by β-glucosidase, obtaining glucose molecules (Lam and Lee, 2015).

Hemicellulose is principally formed by xylose, galactose, mannose, and other sugars. They are joined by β-(1–4) and β-(1–3) linkages, which are cleaved by different enzymes such as xylanases, xilases, α-l-arabinofuranosidase, and β-glucosidase and converting them into monomers. For starch and glycogen, their α-(1–4) D-glucosidic linkages are hydrolysed by α-amylase in a process known as liquefaction. Maltodextrin is the obtained product, which is composed of oligosaccharides with three or more α-(1–4)-linked D-glucose units. After this, the saccharification takes place when maltodextrin is converted into simple reducing sugars by amyloglucosidase. This process acts on both α-(1–4) and α-(1–6) D-glucosidic linkages.

Enzymatic hydrolysis has many advantages over chemical hydrolysis, including mild operation conditions (with subsequent lower energy requirements), higher selectivity and biological specificity (leading to higher conversion yields and lower by-product formation), and easier scale-up. However, it also has major drawbacks, such as enzyme cost and difficult recovery, which could make the process economically unfeasible. The process effectiveness primarily depends on operational parameters such as temperature, pH, time, and enzymes type and concentration, and an optimization of the different parameters must be done for obtaining maximum yields and reducing costs (Choi et al., 2010).

Many authors have investigated the enzymatic hydrolysis of microalgae biomass. For example, Ho et al. (2013) conducted enzymatic hydrolysis at 200 rpm and 45°C with 20 g L^{-1} of *C. vulgaris* (51% of initial carbohydrates, 93.1% of them being glucose). The enzyme mixture consisted of endoglucanase (0.65 U mL^{-1}), β-glucosidase (1.50 U mL^{-1}), and amylase (0.09 U mL^{-1}), and the biomass-to-enzyme ratio was 10 g mL^{-1}. They reported 0.461 $g_{glucose} g_{algae\ dw^{-1}}$ (~97%) after 48 h. Ho et al. compared these results with those obtained by dilute acid hydrolysis at 1% H_2SO_4, 121°C, 20 min, and 50 g L^{-1} of biomass. The glucose concentration achieved was 23.6 g L^{-1} (~100%), a similar conversion yield to that obtained from enzymatic hydrolysis.

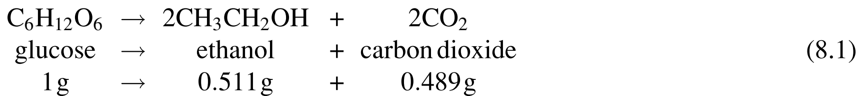
Choi et al. (2010) applied enzymatic hydrolysis (5% solid-to-liquid ratio, 0.005% (v/w) α-amylase, 0.2% (v/w) amyloglucosidase) to *C. reindhardtii* UTEX 90 (59.7% of initial carbohydrates). The process was divided into a liquefaction and a saccharification step. Liquefaction was at 90°C, 30 min, and pH 6, and the optimal saccharification conditions were 55°C, 45 min, and pH 4.5. They reported a glucose yield of 0.561 g $g_{algae\ dw^{-1}}$, which accounts for a 94% conversion of initial glucose.

Kim et al. (2014) studied the effect of enzymatic hydrolysis at 1% (w/v) of microalgae using two separate enzymes: cellulase (Celluclast 1.5 L) and pectinase (Pectinex SP-L) (whose activities were 0.122 FPU $mg_{protein^{-1}}$ and 240 UI $mg_{protein^{-1}}$), respectively. They added 1.88 mg protein per gram of algal biomass working at 50°C, 200 rpm, pH 4.8, 72 h) on bioethanol production from *C. vulgaris* (22.4% of total carbohydrates), achieving only 10% of sugar conversion with cellulase but 45% of sugar release yield with pectinase, hence liberating 0.1 $g_{glucose} g_{algae\ dw^{-1}}$. Furthermore, Mahdy et al. (2016) worked with *C. vulgaris* grown in urban wastewater (39.6% of carbohydrates and 33.3% of proteins). They applied two different enzymes separately: alcalase 2.5 L (0.585 AU $g_{dw^{-1}}$) and Viscozyme (36.3 FBG $g_{dw^{-1}}$) for protein and carbohydrate solubilizations, respectively. The enzymatic hydrolysis was carried out with 3.2% (w/v), pH at 8 (alcalase) and 5.5 (Viscozyme), 50°C and 3 h. The pH was adjusted on demand during the process. They reported organic matter hydrolysis efficiency of 54.7% for proteins (alcalase) and 28.4% for carbohydrates (Viscozyme).

8.6 Fermentation processes

Fermentation processes principally consist of the conversion of the monomeric sugars obtained in previous steps into alcohols. In the case of bioethanol, sugars in the absence of oxygen are commonly converted by *Saccharomyces* and *Zymomonas*

(De Farias Silva and Bertucco, 2016). The maximum ethanol production achieved from glucose is 0.511 $g_{ethanol}\ g_{glucose^{-1}}$ as can be seen in Eq. (8.1) showing the stoichiometry of the reaction:



$$\begin{array}{cccccc} C_6H_{12}O_6 & \rightarrow & 2CH_3CH_2OH & + & 2CO_2 \\ \text{glucose} & \rightarrow & \text{ethanol} & + & \text{carbon dioxide} \\ 1\,g & \rightarrow & 0.511\,g & + & 0.489\,g \end{array} \tag{8.1}$$

Fermentation can be performed in two different ways: with separated hydrolysis and fermentation (SHF) and simultaneous hydrolysis and fermentation (SSF) in the same vessel. For bioethanol production from microalgae biomass, more literature reporting SHF than SSF can be found (Table 8.4) (Lam and Lee, 2015).

For biobutanol, the traditional process consists on the transformation of sugars into a mixture of acetone, butanol, and ethanol (ABE); *Clostridia* genus is the most common microorganism. The typical ratio of products of this process is 3:6:1 (ABE), making butanol the most relevant product (Bellido et al., 2014).

An important issue on fermentation processes is the presence of degradation compounds (by-products) that could inhibit the bioalcohol production. Weak acids, furanic, and/or phenolic compounds could be generated during the pretreatment step and/or enzymatic hydrolysis depending on their operational conditions. They lead to inhibition processes and other negative effects on the fermentation process depending on the by-products concentration. For example, 5-hydroxymethylfurfural and furfural (furanic compounds) can inhibit cell growth, damaging the DNA, whereas phenolic compounds modify the membrane permeability, provoking the loss of intracellular components when affecting the enzymatic pathways. In general the particular effect of each degradation compound is linked to its structure and hydrophobicity. Currently, most of the utilized yeasts or bacteria are genetically modified to avoid some of these problems (Monlau et al., 2014).

8.6.1 Bioethanol production by SHF

The SHF process is usually employed in research to study and optimize the operational conditions (pH, temperature, and time) of both stages. It aids in determining the different mechanisms involved in the process and the influence showed by various parameters as well as performing continuous fermentation with cell recycling. However, the operational procedure has some disadvantages. The process is more difficult and expensive than that of SSF due to the necessity of using a higher enzyme concentration and low solid loading to achieve an acceptable ethanol yield. Additionally, the long times required could cause a substrate's contamination by microbes (Sirajunnisa and Surendhiran, 2016).

Ho et al. (2013) fermented the enzymatic and acid dilute hydrolysates of *C. vulgaris* by SHF. Both hydrolysis conditions were described in Section 8.5, but in this case they used different enzyme concentrations (endoglucanase: 0.61 U mL^{-1}, β-glucosidase: 0.30 U mL^{-1}, amylase: 0.75 U mL^{-1}), achieving 7.78 g L^{-1} of glucose concentration in this enzymatic hydrolysate. Fermentations were conducted with

Table 8.4 **Bioethanol production from different microalgae biomass via fermentation processes**

Microalgae species	Method	Fermentation	Yeast/Bacteria	Initial biomass concentration (g/L)	Total carbohydrates (%)	Sugar release yield (g/g algae)	Ethanol (g/L)	Ethanol yield (g/g algae)	References
Chlorella vulgaris	Acid	SHF	*Zymomonas mobilis*	50	51	0.472	11.66	0.233	Ho et al. (2013)
Chlorella vulgaris	Enzymatic	SHF	*Zymomonas mobilis*	20	51	0.389	3.55	0.178	Ho et al. (2013)
Chlamydomonas reindhardtii UTEX 90	Enzymatic	SHF	*Saccharomyces cerevisiae*	50	60	0.561	11.73	0.235	Choi et al. (2010)
Chlorella vulgaris	Enzymatic	SHF	*Saccharomyces cerevisiae*	10	22	–	0.60	0.070	Kim et al. (2014)
Chlorella vulgaris	Enzymatic	SSF	*Zymomonas mobilis*	20	51	–	4.27	0.214	Ho et al. (2013)
Chlamydomonas fasciata	Enzymatic	SSF	*Saccharomyces cerevisiae*	100	–	–	19.40	0.194	Asada et al. (2012)

bacterium *Zymomonas mobilis* ATCC 29191 (inoculum size of 10%) at 30°C for 12 h. The authors reported ethanol concentrations of 3.55 g L^{-1} (0.178 g ethanol $g_{algae\ dw^{-1}}$) for enzymatic hydrolysis and 11.6 g L^{-1} of ethanol (0.233 g $g_{algae\ dw^{-1}}$) for acid hydrolysis, showing no generation of inhibitor compounds during the acid hydrolysis. In another study, Choi et al. (2010) fermented the liquid obtained after the enzymatic hydrolysis (5% v/v) of *C. reindhardtii* (59.7% of total carbohydrates) with *Saccharomyces cerevisiae* S288C, 30°C, 160 rpm for 40 h, obtaining 11.73 g L^{-1} of ethanol. Furthermore, Harun et al. (2011) applied alkali pretreatment (0.75% (w/v) of NaOH, 120°C, 30 min) in the bioethanol production from *Chlorococcum* sp. (around 44% carbohydrates). The fermentation was conducted at 30°C, 200 rpm for 72 h using *S. cerevisiae*. The glucose yield obtained was 0.350 g $g_{algae\ dw^{-1}}$ with a bioethanol production of 0.26 g ethanol $g_{algae\ dw^{-1}}$, but the researchers did not explain such high fermentation yield results. Kim et al. (2014) fermented the enzymatic hydrolysate (1%, w/v) of *C. vulgaris* (16% of total carbohydrates) with *S. cerevisiae* (pH 5, 30°C, 48 h), reaching 89% of fermentation yield and 0.6 g L^{-1} of ethanol concentration.

8.6.2 Bioethanol production by SSF

The SSF method has some advantages over SHF such as lower cost and lower global processing time. The SSF method requires just one single reactor, so the material costs are lower than with the SHF reactor, and the reaction process is simplified. Nevertheless, there are some drawbacks to the SSF method: enzyme and yeast recoveries are difficult for this process, creating a challenge when scaling it up. Performing both saccharification and fermentation at the same time also makes the process optimization more difficult (Sirajunnisa and Surendhiran, 2016).

Some studies showed higher bioethanol yield for SSF than for SHF. For example, Ho et al. (2013) compared their previously cited SHF experiments with SSF results. The SSF was carried out at 30°C, 60 h with 20 g L^{-1} of biomass concentration in acetate buffer solution (pH 6.0), identical enzymatic cocktails, and *Z. mobilis* inoculum. They reported an ethanol concentration of 4.27 g L^{-1} (0.214 g ethanol $g_{algae\ dw^{-1}}$), which represented slightly higher values than SHF with enzymatic hydrolysis but close to yields of SHF with acid hydrolysis. In addition, Asada et al. (2012) performed SSF with sonicated (30 min) and untreated *Chlamydomonas fasciata* (43.5% of initial carbohydrates). Experiments were conducted at 100 g L^{-1} of microalgae concentration using glutase-AN enzyme (0.1%, w/v) and *S. cerevisiae* at 100 rpm, 40°C for 30 h. Asada et al. obtained 19.4 g ethanol L^{-1} from pretreated samples compared to only 6.94 g ethanol L^{-1} from the untreated biomass.

8.6.3 Biobutanol production

Biobutanol produced from microalgae biomass is emerging as an advanced biofuel and is expected to replace bioethanol at some point. Nevertheless, biobutanol fermentation is less efficient and productive due to the remarkable inhibitory effect of the degradation compounds generated in the previous steps and the single species used for biobutanol (i.e., *Clostridium* spp.) that produces considerable amounts of other

compounds (acetone, bioethanol, and organic acids) (Chen et al., 2015). When the fermentation is controlled, the theoretical maximum ABE yield is $0.41\,g\,g_{sugar^{-1}}$ lower than the $\approx 0.5\,g\,g_{sugar^{-1}}$ for bioethanol, obtaining CO_2 and H_2 as single by-products. Biobutanol production from microalgae starch is as easy as bioethanol production since *Clostridium* spp. are saccharolytic. Nevertheless, limited research work exists on biobutanol production from microalgae cellulose (Chen et al., 2013). Some work about the production of biobutanol from microalgae with a starch-cellulose mix has been reported. Ellis et al. (2012) performed ABE fermentation using microalgae biomass cultivated with wastewater with *Clostridium saccharoperbutylacetonicum* N1–4. They worked at 10% (w/v) of pretreated algae. The fermentation of algae pretreated with acid and basic chemicals resulted in only $2.74\ g\,L^{-1}$ of total ABE whereas applying enzymatic hydrolysis (xylanases and cellulases) obtained $9.74\,g\,ABE\,L^{-1}$. The highest total ABE production yield and productivity were obtained using enzymatic hydrolysis ($0.311\ g\,g^{-1}$ and $0.102\ g\,L^{-1}\,h^{-1}$, respectively).

8.7 Conclusions

Bioethanol production from microalgae biomass has been proposed as an innovative alternative to substitute fossil fuel sources. Unlike other renewable sources (e.g., lignocellulosic materials), microalgae biomass has no lignin, which makes the carbohydrate extraction process easier and eventually it should help to develop cleaner and safer bioethanol production processes. Carbohydrates in microalgae can be present in a variety of forms (cellulose, starch, and/or glycogen) and located in different regions of the cells (inner, inside, outside). Carbohydrate type, location, and concentration will strongly depend on cultivation and operation conditions with concentrations ranging from 15% to 50%. Several steps must be applied to obtain bioethanol from this biomass. First, different methods can be employed to disrupt the cell wall and release the carbohydrates such as physical-mechanicals, chemicals, and/or a combination of them. After that, enzymatic hydrolysis could be required to convert the carbohydrates into simple sugars. Finally, a yeast or bacteria fermentation stage is performed to transform these sugars into ethanol. However, it is imperative that the principal parameters of these different steps should be optimized during the bioethanol production before industrial implementation, and more research on economic and life cycle analysis is needed to ensure the economic feasibility of the process.

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