Chapter 12
Biofuels from Microalgae: Biomethane

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Abstract The high cost of axenic microalgae cultivation in photobioreactors limits nowadays the potential uses of microalgal biomass as a feedstock for the production of biodiesel or bioethanol. In this context, microalgae-based wastewater treatment (WWT) has emerged as the leading method of cultivation for supplying microalgae at low cost and low environmental impacts, while achieving sewage treatment. Nonetheless, the year-round dynamics in microalgae population and cell composition when grown in WWTPs restrict the use of this low-quality biomass to biogas production via anaerobic digestion. Although the macromolecular composition of the microalgae produced during wastewater treatment is similar to that of sewage sludge, the recalcitrant nature of microalgae cell walls requires an optimisation of pretreatment technologies for enhancing microalgae biodegradability. In addition, the low C/N ratio, the high water content and the suspended nature of microalgae suggest that microalgal biomass will also benefit from anaerobic co-digestion with carbon-rich substrates, which constitutes a field for further research. Photosynthetic microalgae growth can also support an effective CO_2 capture and H_2S oxidation from biogas, which would generate a high-quality biomethane complying with most

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international regulations for injection into natural gas grids or use as autogas. This book chapter will critically review the most recent advances in biogas production from microalgae, with a special focus on pretreatment technologies, co-digestion opportunities, modelling strategies, biogas upgrading and process microbiology.

Keywords Anaerobic co-digestion · Biogas upgrading · Microbiology Modelling · Pretreatments

1 Introduction

During the last decade, microalgae production and bioconversion have been widely investigated for bioenergy generation purposes. Nonetheless, energy and life cycle assessments of theoretical and pilot-scale studies have consistently shown that such technology is only feasible if microalgae are grown in open ponds fed with wastewater (Sialve et al. 2009). In this context, high rate algal ponds (HRAPs) have been proved efficient in removing organic matter and nutrients from contaminated effluents (Park et al. 2011), and cost-effective alternatives when compared to activated sludge processes (no external input of aeration is required due to the natural occurrence of photosynthesis).

The microalgae-bacteria biomass produced in such systems may be valorised through anaerobic digestion (AD) with the concomitant production of biogas. This process is already well known and has long been used to produce bioenergy from organic residues such as sewage sludge, agricultural and industrial by-products. In fact, AD may convert microalgae-based wastewater treatment plants (WWTPs) into net energy producers by converting methane into heat and electricity that may be subsequently used in biomass pretreatment and wastewater biodegradation (Passos and Ferrer 2014). Additionally, the mineralisation of microalgae containing organic nitrogen and phosphorus may convert microalgae into a stabilised biosolid fertilizer (Solé-Bundó et al. 2017).

Nonetheless, this technology platform has some bottlenecks that hinder its viability at full-scale. The main issues are: (i) low microalgae production rates due to carbon or light limitation, (ii) costly biomass concentration and (iii) slow biodegradability in anaerobic digesters. Some of these challenges may be overcome by applying pretreatment or co-digestion technologies. Pretreatment can be used to enhance microalgae anaerobic biodegradability by weakening or disrupting microalgae cell wall structure; co-digestion improves the process biogas yield by improving the organic loading rate while controlling ammonia concentration. On the other hand, mathematical models and reactor design and operation strategies need to be carefully reviewed for a better understanding and optimisation of process performance. Finally, the biogas produced during the AD of microalgae should be upgraded prior to its combustion on-site, injection into natural gas grids or used as autogas.

This chapter aims at presenting and discussing the main topics involved in microalgae AD, i.e. the microbiology involved, pretreatment technologies, co-digestion with other substrates, design and operational considerations, process modelling and biogas upgrading to biomethane.

2 The Role of Microbiology in the Anaerobic Digestion of Microalgae

AD of microalgae is a spontaneous process in which organic matter from microalgal cells is converted to biogas through reactions catalysed by naturally occurring microorganisms. Like most biological processes, AD is affected by a variety of factors such as "substrate type", environmental, physical, biological and chemical conditions. Microalgal biomass is composed mainly of organic compounds (mostly lipids, carbohydrates and protein), as well as nitrogen, phosphorus and oligonutrients such as zinc, cobalt and iron. The average composition of microalgae can be expressed as $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Grobbelaar 2004). The content of proteins, lipids and carbohydrates in microalgae is strongly species dependent (Table 1) and varies from 6 to 52%, from 7 to 23% and from 5 to 23%, respectively (Brown et al. 1997).

Two of the most important factors determining the methane yield in anaerobic digestion of microalgal biomass are the composition of microalgae cell wall and its contribution to the total cell mass. Cell wall composition is recognised as the limiting factor in hydrolysis of microalgae (Chen and Oswald 1998. Microalgae cell wall comprises 12–36% of total cell mass (w/w) (Table 2) and may contain biopolymers (e.g. algaenan, cellulose, sporopollenin, glucosamine, proline and carotenoids) and/or structures (such as trilaminar outer wall or trilaminar sheath—TLS) that are resistant to anaerobic degradation (Kadouri et al. 1988; Brown 1997; Derenne et al. 1992; Gelin et al. 1997; Okuda 2002; Simpson et al. 2003). Cell walls recalcitrant to microbial attack may prevent microalgal intracellular organic

Table 1 Gross composition of several microalgae species

Microalgae species	Proteins (%)	Lipids (%)	Carbohydrates (%)
Euglena gracilis	39–61	14–20	14–18
Chlamydomonas reinhardtii	48	21	17
Chlorella pyrenoidosa	57	2	26
Chlorella vulgaris	51–58	14–22	12–17
Dunaliella salina	57	6	32
Spirulina maxima	60–71	6–7	13–16
Spirulina platensis	46–63	4–9	8–14
Scenedesmus obliquus	50–56	12–14	10–17

Adapted from Sialve et al. (2009)

content from being converted to biogas, which affects the final methane yield. However, a variety of pretreatments (below discussed) have been shown to be effective at breaking microalgae cell walls and increasing methane yield (Angelidaki and Ahring 2000; Alzate et al. 2012).

Cultivation of microalgae under nitrogen deficiency is "well-known" to stimulate lipid accumulation (Chisti 2007). Theoretically, the higher the lipid content of microalgae cells, the higher their calorific value and hence the higher their methane yield. However, a high lipid content does not usually correlate with a high methane yield. Therefore, the content of inert organic matter, rather than the content energy-rich macromolecules, is believed to have a stronger impact on the final methane yield (González-Fernandez et al. 2012).

The high content of proteins observed in several microalgae species results in high concentrations of ammonia nitrogen during anaerobic degradation. Ammonia

Table 2 Cell wall composition of microalgae

Microalgae species	Cell wall (% w/w)	Cell wall composition (%)			References
		Carbohydrates	Proteins	c.n.i.[a]	
Chlorella vulgaris (F)	20.0	30.00	2.46	67.54	Abo-Shady et al. (1993)
Chlorella vulgaris (S)	26.0	35.00	1.73	63.27	Abo-Shady et al. (1993)
Kircheriella lunaris	23.0	75.00	3.96	21.04	Abo-Shady et al. (1993)
Klebsormidium flaccidum	36.7	38.00	22.60	39.40	Domozych et al. (1980)
Ulothrix belkae	25.0	39.00	24.00	37.00	Domozych et al. (1980)
Pleurastrum terrestre	41.0	31.50	37.30	31.20	Domozych et al. (1980)
Pseudendoclonium basiliense	12.8	30.00	20.00	50.00	Domozych et al. (1980)
Chlorella Saccharophila	–	54.00	1.70	44.30	Blumreisinger et al. (1983)
Chlorella fusca	–	68.00	11.00	20.00	Blumreisinger et al. (1983)
Chlorella fusca	–	80.00	7.00	13.00	Loos and Meindl (1982)
Monoraphidium braunii	–	47.00	16.00	37.00	Blumreisinger et al. (1983)
Ankistrodesmus densus	–	32.00	14.00	54.00	Blumreisinger et al. (1983)
Scenedesmus obliquus	–	39.00	15.00	46.00	Blumreisinger et al. (1983)

[a]c.n.i. stands for content not identified

is highly permeable through cell membranes and can affect methane yields due to ammonia inhibition. The acclimation period, substrate composition and operating conditions typically determine the inhibitory concentrations of ammonia, which can vary from 0.05 to 2 g/L (Rajagopal et al. 2013). Thermophilic conditions enhance the inhibition effect (Sialve et al. 2009). In this context, methanogenic communities can acclimate to high concentrations of ammonia, increasing the inhibition threshold level, even if methanogenic productivity remains low.

3 Pretreatments for Increasing the Anaerobic Biodegradability

The conversion of microalgae into biogas is often limited by the hydrolysis step of the AD process. In the 1950s, researchers already noticed that microalgae remained intact after AD in a reactor operating at 30 days of hydraulic retention time (HRT) (Golueke et al. 1957). This phenomenon also occurs when biodegrading other complex organic substrates, such as activated sludge and lignocellulosic biomass, in which organic compounds have low bioavailability and/or low biodegradability. This bottleneck may be overcome by applying a previous pretreatment step, which is already the case in full-scale WWTPs treating sewage sludge or in the agroindustrial field. Overall, biomass pretreatment methods aim at increasing organic matter solubilisation and, therefore, making those compounds more readily available to the anaerobic bacteria present in the digester, which would ultimately increase the process rate and the methane yield (Passos et al. 2014a).

Particularly, the main reason why microalgae have slow and/or low biodegradability is due to the nature of their cell wall structure and composition. Most species have a complex cell wall composed of recalcitrant components, especially those grown in open ponds treating wastewater. Nonetheless, the characteristics of these cell walls may vary depending on the strain and environmental/operational conditions. Species with a glycoprotein-based, frustule-covered, or a bacterial-like peptidoglycan cell walls, are more sensitive to disruption with pretreatment techniques than those with silica- or polysaccharide-based cell walls (Bohytskyi et al. 2014). The main constituents of microalgae biomass are carbohydrates, proteins, lipids, carotenoids and lignin. Nonetheless, most of them are polysaccharides, e.g. cellulose, hemicellulose, chitin/chitosan-like molecules, pectin and alginate. A recent study found that, although proteins, lipids and a considerable amount of carbohydrates were present in the cell walls of refractory microalgae species, microalgae resistance was not correlated to the presence of a unique monomer. The authors concluded that the responsible compounds were most likely to be sporopollenin, lignin-like materials and heteropolysaccharides (Montingelli et al. 2015). However, it is hypothesised that the cross-link of these compounds into a complex network building layers around the cell could eventually work as a barrier to anaerobic microbial community (Klassen et al. 2016).

Pretreatment techniques may be classified into four main categories: mechanical, thermal, chemical and biological methods. These methods are based on different mechanisms and, therefore, support different disruption efficiencies. For instance, mechanical techniques, such as microwave, ultrasound and ball-milling, act by reducing the particle size and increasing the superficial contact area; while biological pretreatments act by inducing an enzymatic breakdown of complex molecules. In a study comparing different techniques, physical pretreatments (i.e. thermal and ultrasound) showed the highest effectiveness in protein solubilisation, which was mediated by the release of alogenic organic matter and cell wall breakage, while enzymatic pretreatments increased carbohydrate solubilisation, which was mediated by the biodegradation of cell wall compounds rather than by cell disruption (Ometto et al. 2014). In this experiment, the highest biogas increase in batch tests was obtained for enzymatic pretreated microalgae (270% increase).

Most studies up-to-date were conducted using batch experiments. These tests are mainly used for comparing pretreatments and/or pretreatment conditions. However, continuous experiments with acclimated microorganisms are needed for validating and quantifying the potential methane yield and for estimating the energy balance of the process. Among the studies published so far, most of those dealing with continuous AD of microalgae evaluated the effect of thermal pretreatment. The results reported showed increases from 32 to 108% compared to non-pretreated microalgae (ranging from 0.12 to 0.27 L CH_4/g VS) (Table 3). The best results were obtained during microalgae thermal pretreatment at 75–95 °C for 10 h (70% increase) (Passos and Ferrer 2014) and 120 °C for 2 h (108% increase) (Schwede et al. 2013). Moreover, the energy balance calculations showed that after applying a low-temperature pretreatment at 75 °C, the energy balance shifted from neutral to positive with a 2.7 GJ net energy production per day (Passos and Ferrer 2014). In fact, most recent reviews in microalgae pretreatment concluded that thermal pretreatment is the optimal method, by combining the highest methane improvement and the lowest energy input (Jankowska et al. 2017; Passos et al. 2014a, b; Rodriguez et al. 2015).

Additionally, enzymatic pretreatment has recently been the focus of research on microalgae pretreatment. Studies in continuous mode showed increases of 260% in methane yield compared to non-pretreated microalgae, although biomass was highly recalcitrant in this experiment, i.e. 0.05 L CH_4/ g COD (Mahdy et al. 2015). The enzymatic pretreatment of *Scenedesmus* sp. in a first step anaerobic membrane bioreactor (AnMBR) with rumen microorganisms also showed promising results in terms of methane yield (0.203 L CH_4/g COD) and COD removal (70%) (Giménez et al. 2017).

Although many novel pretreatment methods are being investigated, such as pulse electric field, ozonation or solvent addition, the energy and economic aspects for pilot and full-scale viability must be analysed. The main pros and cons of microalgae pretreatment techniques are summarised in Table 4. Thus, energy demand and scalability are major issues when evaluating pretreatment viability. Although thermal pretreatment seems advantageous, biomass thickening or dewatering is crucial. On the other hand, despite thermochemical pretreatments have

Table 3 Microalgae pretreatment for improved AD in continuous reactors

Microalgae species	Pretreatment conditions	AD conditions	Methane yield increase	References
Scenedesmus sp. and *Chlorella* sp.	Thermal: 100 °C, 8 h	CSTR[a]: 3.7% TS, 28 days HRT[b]	33% (0.270 L CH_4/g VS)	Chen and Oswald (1998)
Scenedesmus sp., *Monorraphidium* sp. and diatoms biomass	Thermal: 75 and 95 °C, 10 h	CSTR: 37 °C, 0.7 g VS/Ld, 20 days HRT	70% (0.180 L CH_4/g VS)	Passos and Ferrer (2014)
Pediastrum sp., *Micractinium* sp. and *Scenedesmus* sp.	Thermal: 60 °C, 2–6 h	AVR[c]: 20 °C, 1.2 g VS/Ld, 91 days SRT[d]	32% (0.136 L CH_4/g VS)	Kinnunen et al. (2014)
Nannochloropsis salina	Thermal: 100–120 °C, 2 h	CSTR: 38 °C, 2.0 g VS/Ld, 120 days HRT	108% (0.130 L CH_4/g VS)	Schwede et al. (2013)
Oocystis biomass	Thermal: 130 °C, 15 min	CSTR: 37 °C, 0.7 g VS/Ld, 20 days HRT	42% (0.120 L CH_4/g VS)	Passos and Ferrer (2015)
Chlorella vulgaris	Thermal: 120 °C, 40 min	CSTR: 35 °C, 1.5 g COD/Ld, 15 days HRT	48% (0.126 L CH4/g COD)	Sanz et al. (2017)
Scenedesmus sp., *Monorraphidium* sp. and diatoms biomass	Microwave: 70 MJ/kg VS, 26 g TS/L	CSTR: 35 °C, 0.8 g VS/Ld, 20 days HRT	60% (0.272 L CH_4/g VS)	Passos et al. (2014b)
Chlorella vulgaris	Enzymatic: protease (0.585 UA), 65 g TS/L	CSTR: 35 °C, 1.5 g COD/Ld, 20 days HRT	260% (0.128 L CH_4/g COD)	Mahdy et al. (2015)
Scenedesmus sp.	Enzymatic: rumen microorganisms fermenter	AnMBR[e]: 38 °C, 0.2 g COD/Ld, 31 days HRT, 100 days SRT	0.203 L CH_4/g COD	Giménez et al. (2017)

Notes [a]CSTR stands for complete stirred tank reactor, [b]HRT stands for hydraulic retention time, [c]AVR stands for accumulating volume reactor, [d]SRT stands for sludge retention time, and [e]AnMBR stands for anaerobic membrane bioreactor

supported positive microalgae biodegradability increases, further studies should evaluate the risk of contamination in continuous bench and pilot-scale reactors. An alternative cost-effective microalgae pretreatment method may be the use of environmentally friendly and low-cost chemicals such as lime (CaO). A recent study found that the methane yield increased by 25% in BMP tests after pretreating microalgae at 72 °C with CaO (Solé-Bundó et al. 2017). Biological pretreatments constitute another promising pretreatment technology. Experiments conducted so far have still not elucidated the best pretreatment conditions, resulting in lower biogas production increases compared to thermal and thermochemical methods. In

Table 4 Comparison of pretreatment methods for increasing microalgae anaerobic biodegradability (Passos et al. 2014a)

Pretreatment	Control parameters	Anaerobic biodegradability increase	Pros	Cons
Thermal (<100 °C)	Temperature; exposure time	√√	Lower energy demand; scalability	High exposure time
Hydrothermal (>100 °C)	Temperature; exposure time	√√	Scalability	High heat demand; need for thickened or dewatered biomass; risk of formation of refractory compounds
Thermal with steam explosion	Temperature; exposure time; pressure	√√√	Scalability	High heat demand; Need for thickened or dewatered biomass; risk of formation of refractory compounds Investment cost
Microwave	Power; exposure time	√√	–	High electricity demand; scalability; need for biomass dewatering
Ultrasound	Power; exposure time	√	Scalability	High electricity demand; need for biomass dewatering
Chemical	Chemical dose; exposure time	√	Low energy demand	Chemical contamination; risk of formation of inhibitors; high cost
Thermochemical	Chemical dose; exposure time; temperature	√√	Low energy demand	Chemical contamination; risk of formation of inhibitors; high cost
Enzymatic	Enzyme dose; exposure time; pH, temperature	√	Low energy demand	Cost, sterile conditions

addition, purified enzymes may be expensive and jeopardise the economic viability of the process. However, this limitation may be overcome via enzyme production through other microorganisms, via enzyme expression through the microalgae cells to be digested and via in situ production of hydrolytic enzymes by inoculated living bacteria or fungi (Klassen et al. 2016).

Finally, future research should focus on investigating the mechanisms underlying microalgae cell wall damage and/or disruption with pretreatments, since the analysis of organic matter solubilisation has been shown insufficient to predict the increase in methane yields. The determination of soluble macromolecules, microscopic images and microbiology analyses is important for better understanding how, where and in which scale pretreatments affect microalgae cell structure and which compounds become more readily available. Moreover, it is crucial to conduct experiments in continuous mode and in pilot and full-scale reactors for evaluating the process performance.

4 Anaerobic Co-digestion of Microalgae

AD of raw microalgae or microalgae residues after the generation/extraction of value-added products (i.e. lipids, ethanol and hydrogen) is typically characterised by low methane yields and the occurrence of ammonia inhibition. Despite these limitations, AD is still regarded as a key technology to maximise resource recovery from microalgae and make algae industry economically feasible. AD also aids the mobilisation the nutrients (N and P) needed for algae cultivation (Ward et al. 2014). Anaerobic co-digestion, the simultaneous digestion of two or more substrates, is an established and cost-effective option to overcome the drawbacks of mono-digestion and boost the biogas production of AD plants (Mata-Alvarez et al. 2014). Besides improving the feasibility of AD plants, co-digestion also allows treating several wastes in a single facility and "share/reduce" treatment costs (Neumann et al. 2015).

Algae have been successfully co-digested with a large range of co-substrates such as sewage sludge, animal manures, food waste, energy crops, glycerol, paper waste and fat, oil and grease (FOG). Although the improvement of the methane production is mainly a consequence of the increased organic loading rate (OLR) rather than to the occurrence of synergisms during AD, microalgae have been primarily co-digested with carbon-rich co-substrates, which allows increasing the digester OLR while controlling ammonia concentration. Several studies have optimised the co-substrate dose by balancing the feedstock C/N ratio with optimum values for algae co-digestion ranging between 12 and 27 (Ehimen et al. 2011; Fernández-Rodríguez et al. 2014). However, optimising co-substrate selection and dosage based on the C/N ratio is an oversimplification since this approach does not take into account the characteristics of each co-substrate (Astals et al. 2014; Herrmann et al. 2016). The maximum dose of some co-substrates such as glycerol and FOG is limited by secondary inhibitory mechanisms, while the deficiency of alkalinity or essential nutrients limits the dosage of energy crops and paper waste

(Schwede et al. 2013; Zhong et al. 2013). The maximum dosing rate of self-sufficient co-substrates such as food waste or sewage sludge is typically limited by the anaerobic digestion plant capacity and co-substrate availability. Regardless of the co-substrate, anaerobic co-digestion stands as a suitable option to reach OLR higher than 2 g VS/L/d in algae digesters, since the operation of algae mono-digesters at OLR higher than 2 g VS/L/d has resulted in inhibitory ammonia concentrations and caused the accumulation of volatile fatty acids (VFAs) (i.e. higher risk of process failure) or even process failure (Yen and Brune 2007; Park and Li 2012; Herrmann et al. 2016).

The integration of algae cultivation in WWTP to substitute the conventional activated sludge reactor, to treat the anaerobic digestion supernatant, or to polish the WWTP final effluent followed by their co-digestion with sewage sludge is attracting a lot of attention (Sahu et al. 2013; Beltran et al. 2016; Peng and Colosi 2016). The cultivation of algae on anaerobic digestion supernatant (diluted or pretreated) is of special interest since it (1) reduces the nutrient load to the headworks, which represents about 20% of the WWTP nutrient load; (2) mitigates greenhouse gases emissions by using CO_2 from biogas combustion for algae growth; and (3) produces algae as on-site co-substrate, which lowers the uncertainty about co-substrate availability and seasonality (Rusten and Sahu 2011; Yuan et al. 2012). Even though this scenario appears very promising, it remains uncertain if the amount of algae able to grow on digester supernatant is enough to make a significant difference on the WWTP methane production (Hidaka et al. 2017). Conversely, the addition of large amounts of algae (or any other nitrogen-rich co-substrate) should be carefully evaluated since it will increase the digester and supernatant nitrogen concentration. In this regard, Mahdy et al. (2017), who co-digested algae and cattle manure, showed that inoculum acclimation could provide anaerobic digestion stable performance at nitrogen concentrations as high as 4 gNH_4^+-N/L and 700 mgNH_3-N/L. Likewise, Arnell et al. (2016) plant-wide simulation study warned of the impact of co-digesting nitrogen-rich waste on the WWTP water train, e.g. aeration requirement, methanol consumption, effluent quality. Finally, the cultivation of microalgae on pig and cattle manure effluent supernatant, and its subsequent co-digestion, has also been studied with the aim of increasing the methane production and moving the nutrients from the supernatant to the biosolid (Wang et al. 2016a, b; Mahdy et al. 2017).

5 Design and Operational Considerations

Biogas production using microalgae as substrate has been studied since the 1950s. The first report addressing the anaerobic digestion of microalgal biomass was published by Golueke et al. (1957). This early study reported a biogas production of 0.5 m^3/kg of volatile solids of algal biomass. During the last decade, an intensive research has been conducted in order to develop solar energy fixation processes

using microalgae to transform light into chemical energy and anaerobic digestion to transform such biomass into biomethane.

When considering biogas production from microalgae, two scenarios should be considered. The first one relies on coupling biogas production to a microalgae-based biodiesel production process. Microalgae have received great attention as a potential source of oil for biodiesel production due to the ability of certain types of microalgae to accumulate lipids and to the higher biomass productivities achieved when compared with land-based crops (Chisti 2007; Mata et al. 2010; Weyer et al. 2010). When the primary use of microalgae is biodiesel production, the lipids extraction processes employed (usually involving solvents) will generate a "residual" biomass suitable for biogas production. However, recent concerns have been raised by life cycle analyses when considering biodiesel production from microalgae due to potentially low energetic yield when based on traditional technology (Scott et al. 2010; Sialve et al. 2009; Stephens et al. 2010). Indeed, a negative energy balance has been estimated for biodiesel process from microalgae as a result of harvesting and drying steps, which are highly energy intensive (Lardon et al. 2009; Scott et al. 2010). In this context, the production of biogas as a sole fuel using whole microalgae has been proposed. This option would entail a much simpler process, with less and simpler unit operations. However, energy in the form of methane possesses nowadays a low economic value.

Hydrolysis is known to be the rate-limiting step of anaerobic digestion of solid substrates, which is specially the case when using microalgae as a substrate. Thermophilic digestion has been proposed as a way to enhance microalgae biomass hydrolysis and the overall anaerobic digestion performance. The high temperatures applied during thermophilic anaerobic digestion (50–57 °C) accelerate biochemical reactions, increasing both the efficiency of organic matter degradation and the potentially applicable organic loading rates. However, higher degradation and loading rates will increase the concentration of ammonia nitrogen in the digester. Contradictory results have been reported when addressing the thermophilic anaerobic digestion of microalgal biomass (Capson-Tojo et al. 2017; Cea-Barcia et al. 2015; Zamalloa et al. 2012a). Indeed, the benefits of the thermophilic digestion of microalgae still need to be confirmed and most likely, the optimum temperature for anaerobic digestion might be dependent on the microalgae species.

The nitrogen content of microalgae biomass is relevant since ammonia release during anaerobic digestion is expected to be an issue of concern as a result of the above-discussed inhibition of AD. This will be especially critical when oil-extracted microalgae are used as substrate, since lipids extraction increases the proportion of nitrogen per gram of biomass. If anaerobic digestion is performed at solids concentrations over 4–5%, ammonia concentration in digester could reach inhibitory levels for the anaerobic microbial community (Torres et al. 2013). Even though the use of ammonia tolerant inocula may provide conditions for successful operation (Mahdy et al. 2017), measurements need to be taken in order to ensure a stable process performance. In this context, co-digestion of microalgae biomass with carbon-rich substrates or wastes could be an alternative. As previously discussed, indeed, the benefits derived from the co-digestion of microalgae biomass with

glycerol, activated sludge and others wastes have consistently showed (Fernandez-Rodriguez et al. 2014; Herrmann et al. 2016; Neumann et al. 2015).

Continuous stirred tank reactors like those used for sewage sludge digestion or other organic substrates are the most popular bioreactor configuration for the conversion of microalgae biomass into biogas. Indeed, most of the reported studies used that configuration with hydraulic retention times ranging from 20 to 40 days (Jankowska et al. 2017). However, other alternative bioreactor configurations such as UASB reactors have been proposed (Tartakovsky et al. 2015). Unfortunately, the low solids retention times of granular-based reactors may not provide an efficient conversion of microalgae. The use of membrane bioreactors has also been proposed, which represents an interesting opportunity to provide the required solids retention for effective microalgae digestion (Zamalloa et al. 2012b) and to tackle the problem of ammonia inhibition. Hence, medium exchange without biomass washout can be implemented in membrane bioreactors to reduce the toxicity mediated by NH_3 built-up, although the operating costs associated to this operational strategy still need to be evaluated under full-scale implementation.

6 Process Modelling

Process modelling is defined as the mathematical representation of a certain process or system, which could be either based on the underlying mechanisms or phenomena (model-based) or on the experimentally generated input/output data (data-based). Mathematical modelling is being increasingly used as a tool for diagnosis, hypothesis formulation, prototyping, scenarios evaluation, process design and optimisation. Thus, the anaerobic degradation of organic biomass, including microalgae, can be modelled using different models. In this context, the Anaerobic Digestion Model 1 (ADM1) has been the most popular, accepted and applied model in research and industrial applications (Batstone and Keller 2002). Nonetheless, there are plenty of other modelling approaches that have been reviewed in the literature (Batstone 2006; Donoso-Bravo et al. 2011; Tomei et al. 2009).

Regardless of the model used to describe the methane production from microalgae, the most important issue is the proper selection of the model parameters. In the specific case of microalgae, as discussed in the above sections, the disruption of microalgae cell wall is considered the limiting reaction step, especially if non-pretreated microalgae are fed to the anaerobic digester. Therefore, both the disintegration and the hydrolysis coefficients, required in ADM1, have to be carefully estimated. However, it is worth to point out that the elimination of the disintegration step has been recommended due to the fact that the use of a two-hydrolysis step possesses some correlation and identification problems, especially for sewage sludge approaches (Batstone et al. 2015). A description of how the modelling of methane production from microalgae has been addressed in different operation modes is given and discussed below.

(Semi) continuous operation. The ADM1 has been used to represent the AD of microalgae by tweaking the original ADM1 with the inclusion of the *Contois* equation instead of the first-order equation to represent the hydrolysis step (Mairet et al. 2011). The *Contois* equation takes into account both the particulate material and the microbial population responsible for this process, while the first-order equation only considers the particulate substrate concentration. The model outperformed the original ADM1 with experimental data from a digester operating for 140 d. An interesting application in this study was the representation of the semi-continuous feeding mode by considering successive batch reactor operations changing the initial conditions for each daily pulses. Moreover, another study tested the same modified ADM1 above-mentioned in an integrated system of wastewater treatment and AD of microalgae (Passos et al. 2015). In this work, the authors found that an appropriate characterisation of the microalgae composition was of paramount importance in order to have a proper model performance due to population changes over time (in particular, the variations in the inert and organic content of the biomass). In addition, a reduced mechanistic 3-reaction model, obtained after principal component analysis, was developed and calibrated with the ADM1 (Mairet et al. 2012). The model was composed of a double hydrolysis reaction to describe the production of volatile fatty acids and a methanogenic reaction. The performance of this model was quite similar to the same simulation results obtained with the ADM1 model, despite its complexity was much lower.

Batch operation. Batch tests, namely biochemical methane potential (BMP) assays, are widely used to assess the kinetics of biodegradation of different substrate. From this test, many parameters such as the hydrolysis coefficient or the inert fraction of the microalgae may be determined when a proper kinetic expression is used (Donoso-Bravo et al. 2010). The first-order model has been a popular option to draw parameters by fitting the accumulated biogas production (Eq. 1).

$$B(t) = B_{\max}(1 - e^{-k_h * t}) \tag{1}$$

This has been done using as a substrate a residual microalgae (i.e. after lipids extraction) (Neumann et al. 2015) or raw microalgae (Fernández-Rodríguez et al. 2014). Some of the values found up-to-date in literature are shown in Table 5. Moreover, the synergism in the co-digestion of microalgae with waste-activated sludge was assessed by the application of a first-order equation to describe the hydrolysis reaction and the *Monod* equation to model the methanogenesis (Lee et al. 2017). To our knowledge, the *Contois* equation has not been yet used to describe the performance of BMP assays.

Global approaches. Apart from the classic modelling application in continuous or batch mode, other new approaches such as the global WWTP plant-wide model that aims at representing an integrated process have been recently developed. This approach intends to implement a model-based on nonlinear programming to evaluate the best configuration of a microalgae-based biorefinery in which AD is also incorporated (Rizwan et al. 2015).

Table 5 Kinetic parameters from the first-order equation in the AD of microalgae

References	k_H (1/d)	B_{max} ($mLCH_4/gVS$)	Microalgae
Neumann et al. (2015)	0.09	413 (13)	*B. braunii*
Fernández-Rodríguez et al. (2014)	0.49 (0.08)	62	*D. salina*
Lee et al. (2017)	0.07[a]	–	*Chlorella* sp.
Wang et al. (2016a, b)	0.148	180.3	*Chlorella* sp.
Zhen et al. (2016)	0.187	106.9 (3.2)	*Scenedesmus* sp.—*Chlorella* sp.

[a]Modified first-order equation

ADM1 simulation of continuous anaerobic digestion of microalgae: The effect of parameter selection. Figure 1 shows the performance of a virtual anaerobic digester operating in continuous mode with microalgae as a feedstock. The results obtained from experiments investigating the AD of raw and residual microalgae were used to perform the simulations (Fernández-Rodríguez et al. 2014; Neumann et al. 2015). To this aim, the inlet COD was fixed at 50 gCOD/L and the volume of the reactor at 1000 m^3, while the organic loading rate was increased by changing the inlet microalgae flow rate. In addition, the macromolecular composition of the raw microalgae was set at 58, 22 and 20% for proteins, carbohydrates and lipids, respectively (Passos et al. 2015). The simulation considered an inert fraction of the organic matter of 24%, estimated from the BMP results. The macromolecular composition of the residual microalgae was set at 64.5, 31.3 and 4.2% for proteins, carbohydrates and lipids, respectively (adapted from Neumann et al. 2015). In this

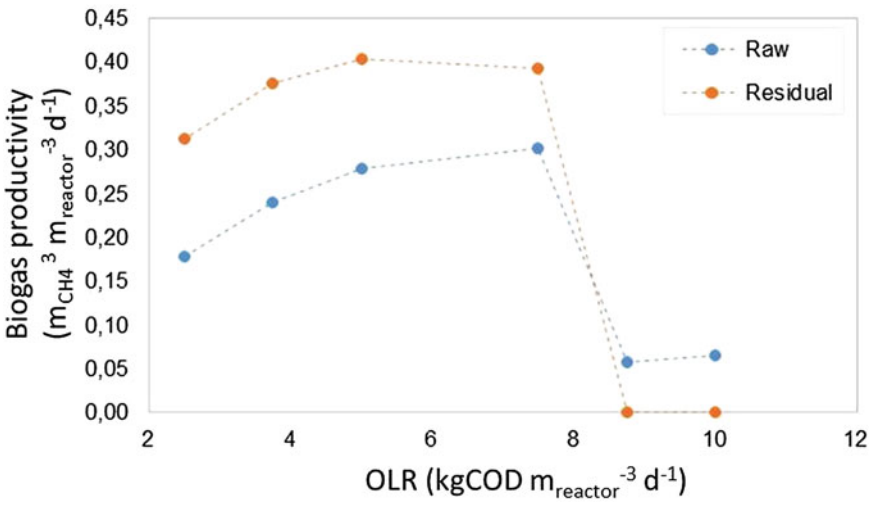

Fig. 1 Simulation of the influence of the organic loading rate on the continuous anaerobic degradation of raw and residual microalgae biomass

case, the simulation considered an inert fraction of the organic matter of 60%, given the amount of methane produced in the BMP test compared to the assay carried out with raw microalgae. The values of the hydrolysis coefficient are shown in Table 5.

The AD of the residual microalgae outperformed the AD of raw microalgae in continuous mode as OLR increased, which may be explained by the low biodegradability of the raw microalgae (Fig. 1). However, methane production in the digester fed with residual microalgae dropped to zero at high OLR values, likely due to the low values of the hydrolytic constant. In contrast, the digester operated with raw microalgae supported a low but stable methane productivity, likely due to the retention of the hydrogenotrophic methane population inside the reactor.

7 Biogas Upgrading to Biomethane

Biogas from the anaerobic digestion of microalgae is typically composed of CH_4 (60–75%), CO_2 (25–30%), H_2S (0–1%), O_2 (0–1%), N_2 (0–4%) and trace levels of NH_3, volatile fatty acids (VFAs) and siloxanes (the latter present in microalgae grown in domestic wastewater) (Alzate et al. 2012). Biogas composition determines the final energy use of this renewable energy feedstock, which ranges from on-site combustion for heat (boilers) or heat/electricity generation (internal combustion engines, turbines, fuel cells), use as a vehicle fuel, and injection into natural grad grids (Bailón and Hinge 2012). In this context, while boilers and internal combustion engines require a removal of H_2S below 0.02–0.1% levels (depending on the manufacturer), micro-turbines and turbines can stand H_2S concentrations in the range of 1–7%. However, the latter require an efficient removal of siloxanes (<0.03–0.1 ppm_v), while internal combustion engines and boilers can cope with concentrations of 5–28 mg Si m^{-3}. Nowadays, the technical requirements for biogas injection into natural gas grids or biogas used as a vehicle fuel are country-specific, although a European draft for biogas quality is currently under approval (Table 6). This entails the need for a biogas-upgrading step prior biogas valorization, which will be stricter when biogas is to be injected into natural gas networks (in the form of biomethane).

Biogas-upgrading technologies can be classified into physical/chemical and biological as a function of the mechanisms governing pollutants removal from biogas. Nowadays, O_2 and N_2 can be only removed by physical/chemical methods (such as membrane separation or low-pressure PSA) (Muñoz et al. 2015), while the removal of CO_2, H_2S, NH_3, VFAs and even siloxanes can be carried using both platform technologies.

Today, the market of CO_2 removal is mainly dominated by water scrubbing (with a 41% of the market share), followed by chemical scrubbing (22%), pressure swing adsorption (21%), membrane separation (10%) and organic solvent scrubbing (6%) (Thrän et al. 2014). Physical/chemical technologies for CO_2 removal from biogas exhibit a high efficiency and robustness at the expenses of high investment and operating costs. Typical CH_4 concentrations in the biomethane

Table 6 Technical specifications for biomethane injection into natural gas grids according to the European draft FprEN 16726

Total S (mg/m^3)	H_2S + COS (mg/m^3)	RSH + Mercaptans (mg/m^3)	O_2 (%)	CO_2 (%)	CO (%)	Volatile Si (mg/m^3)	Amines (mg/m^3)	H_2O (°C) 70 bar	HC (°C) 1–70 bar
20	5	6	0.001–1	2.5–4	0.1	0.1–1	10	−8	−2

produced by the above-mentioned scrubbing, membrane and adsorption technologies range from 95 to 98% (Bauer et al. 2013). However, the CO_2 footprint of these technologies is high as a result of the direct release to the atmosphere of the CO_2 separated and their high energy demand (which represents 3–12% of the energy content present in the raw biogas). Table 7 summarises the fundamentals and the typical design-operating parameters of the main physical/chemical technologies for CO_2 separation from biogas.

Biological CO_2 removal from biogas is still in an early stage of investigation, hydrogenotrophic CO_2 reduction to CH_4 and photosynthetic CO_2 assimilation being the two most promising technologies under scale up. Hydrogenotrophic CO_2 removal, also named power-to-gas, is based on the bioconversion of CO_2 to CH_4 using H_2 as an electron donor and CO_2 as a carbon source and electron acceptor by hydrogenotrophic archaea. Equation 2 describes the stoichiometry of this CO_2 reduction, which can be conducted either directly into the anaerobic digestion (via H_2 supplementation) or in an external bioreactor supplemented with H_2 and biogas:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \Delta nG^0 = -130.7\,\text{kJ/mol} \tag{2}$$

From an economic and environmental viewpoint, hydrogenotrophic CO_2 removal should be based on H_2 produced from water electrolysis using the excess of renewable electricity (i.e. wind power generated during the night). The main limitation of this technology derives from the limited gas–liquid H_2 mass transfer as a result of the low aqueous solubility of this gas (Diaz et al. 2015). On the other hand, photosynthetic CO_2 removal is based on the intensification of the symbiosis between microalgae and quimioautotrophic bacteria at a high pH (=enhancement in the CO_2 and H_2S biogas–liquid mass transfer) in photobioreactors as a platform technology to simultaneously remove CO_2, H_2S, NH_3 and VFAs from biogas at a low energy cost and with a low environmental impact. In these systems, microalgae use the solar energy to fix the CO_2 from biogas via photosynthesis (Meier et al. 2015). Residual nutrients from the effluents of the anaerobic digesters can be used to support microalgae growth, which will significantly reduce the operation cost of the upgrading process and partially mitigate the eutrophication potential of the digestate. This technology has been successfully implemented in open high rate algal ponds interconnected to external absorption columns at 2–3 times lower operating costs than their physical/chemical counterparts (Toledo-Cervantes et al. 2017).

The other major biogas pollutant, H_2S, can be removed using physical/chemical and biological technologies already available at commercial scale (Abatzoglou and Boivin 2009). Adsorption (with and without chemical reaction) and in situ chemical precipitation still represent the two most widely implemented technologies worldwide despite their high operating cost (3.2 and 2.4 cts €/m^3, respectively). Similarly to their CO_2 removal counterparts, these physical/chemical technologies exhibit high efficiencies and a high robustness. Likewise, biotechnologies such as biotrickling filtration and microaerobic anaerobic digestion support high removal efficiencies (>99%) at significantly lower operating cost (1.5 and 0.28 cts €/m^3,

Table 7 Physical/chemical technologies for CO_2 removal from biogas (Bauer et al. 2013; Muñoz et al. 2015)

Technology	Fundamentals	Design parameters	Operational parameters
Water scrubbing	Pressurised water is used for the absorption of CO_2 from biogas in a packed bed. CO_2 separation is based on the higher aqueous solubility of CO_2 compared to that of CH_4 (24 times more soluble)	1 absorption column + 2 stripping columns Concentrations of CH_4 > 96% and of CO_2 < 2%	Operating pressure = 6–10 bar Recycling water flow rates = 0.18–0.23 m^3water/Nm^3_{biogas} Electricity consumption = 0.24 kWh/Nm^3
Chemical Scrubber	Absorption + reaction in solvents based on amines or basic solutions (NaOH, KOH, CaOH, K_2CO_3, etc.)	1 absorption column + 1 stripping column	Operating pressure = 1–2 bar Electricity consumed = 0.13 kWh/Nm^3 Thermal energy for solvent regeneration 0.55 kWh/Nm^3
Organic solvent scrubbing	CO_2 absorption based on polyethylene glycol solvents (Selexol® o Genosorb®) with a 5 times higher CO_2 solubility than water	1 absorption column + 2 desorption column Concentrations of CH_4 = 96–98.5%	Electricity consumed = 0.22 kWh/Nm^3 Thermal energy for solvent regeneration: 0.4–0.51 kWh/Nm^3
Pressure swing adsorption	Selective separation of CO_2 over CH_4 based on a selective adsorption or size exclusion in the adsorbent bed	Adsorbent materials: Activated carbon, silica gel, Zeolites 4 columns operated sequentially Concentrations of CH_4 = 96–98%	Electricity consumed = 0.26 kWh/Nm^3
Membrane separation	Selective permeation of CO_2 and H_2S through semi-permeable membranes	Gas–gas or liquid–gas configurations Single stage or multiple stage configurations	Electricity consumed = 0.26 kWh/Nm^3

respectively) (Gabriel et al. 2013; Muñoz et al. 2015). Among biological methods, photosynthetic H_2S removal is attracting a significant attention based on its simultaneous occurrence during CO_2 capture in algal–bacterial photobioreactors, which will drastically reduce the operating cost of biogas upgrading (Table 8).

Finally, the removal of volatile fatty acids and siloxanes is mainly conducted in conventional adsorption units due to its compact nature and extensive design

Table 8 Technologies for the removal of H_2S from biogas

Technology	Fundamentals	Design parameters	Operational parameters
Adsorption	Adsorption + reaction in an adsorbent packed bed	1 Adsorption column + 1 desorption column Adsorbent: Fe_2O_3, $Fe(OH)_3$ and ZnO	Empty bed residence time = 1–15 min Adsorption capacity of activated carbon: 0.1–0.2 g H_2S/g carbon
Chemical precipitation	Addition to the digester of $FeCl_2$, $FeCl_3$ and $FeSO_4^2$ salts to promote the in situ precipitation of FeS	Levels of H_2S in the treated biogas > 100–150 ppm_v	Dosing ratio = 0.035 kg $FeCl_3$/kg Total solid
Photosynthetic H_2S removal	Aerobic oxidation of H_2S by chemolitotrophic bacteria using the O_2 produced photosynthetically by microalgae in the photobioreactor	H_2S removals > 99%	Liquid to biogas ratio 0.5–2 between the absorption column and the HRAP
Biotrickling filtration	Aerobic or anoxic oxidation of H_2S in a packed bed column containing a biofilm of chemolitotrophic bacteria supplied with nutrients from a recirculating aqueous solution	H_2S removals > 99%	Empty bed residence time = 2–10 min
Microaerobic anaerobic digestion	O_2 dosing in the headspace of the anaerobic digester to support the partial oxidation of H_2S to elemental sulphur that accumulates in the digester headspace	H_2S removals > 99%	Empty bed residence time = 5 h O2/biogas flow rate ratio = 0.3–3%

experience. However, both VFAs and siloxanes are biodegradable molecules and their removal from biogas could be eventually carried out using biotechnologies, which would a priori support a better environmental and economic performance (Accettola et al. 2008).

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