Chapter 6

Challenges for Cost-Effective Microalgae Anaerobic Digestion

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1. Introduction

Microalgae, the common denomination for a broad group of photosynthetic prokaryotes and eukaryotes, are characterized by an efficient conversion of the solar energy into biomass. They are a promising feedstock for the production of third generation biofuels for several reasons:

1. Microalgae photosynthesis allows biological CO_2 fixation, which is expected to mitigate atmospheric CO_2 increase (Amin 2009; Brennan & Owende 2010; Mutanda *et al.* 2011).
2. Microalgae are 10 – 50 times more efficient than plants in terms of CO_2 fixation (Wang *et al.* 2008). Thus, microalgae can fix 1.83 tonnes of CO_2 per 1 tonne of produced microalgae (Chisti 2007).
3. Microalgae can be produced on non-arable areas such as lakes, oceans or deserts, thus reducing competition with food production (Mussgnug *et al.* 2010; Stephens *et al.* 2010). This advantage is a key factor when energy supply is considered in desert zones near oceans.
4. Some microalgae can grow under saline conditions, which strengthen the use of microalgae as feedstock for biofuel production in desert zones near the ocean when freshwater supply is not feasible.

Most of current efforts to take advantage of microalgae as a source of bioenergy are directed to biodiesel production, considering the ability of certain types of microalgae to accumulate lipids under controlled culture conditions. Microalgae biodiesel produced from microalgae lipids also presents technical advantages compared to lignocellulosic biomass based biodiesel.

Biodiesel from microalgae has a higher calorific value (30 and 29 MJ/kg for *C. protothecoides* and *Microcystis aeruginose*, respectively) and lower viscosity and density than plants-based biodiesel (Costa & de Morais 2011). However, the biodiesel yield from algae is rather low compared to biodiesel from lignocellulose energy (Chisti 2007; Sialve *et al.* 2009; Scott *et al.* 2010; Stephens *et al.* 2010). Indeed, with current technology, a negative energy balance was calculated by Lardon *et al.* (2009) when evaluating biodiesel production from *C. vulgaris*, considering biomass drying and further lipid extraction by solvents. During biodiesel production from microalgae, energy consumption associated with culture mixing and pumping, lipid extraction, nutrients addition, drying is of particular importance (Scott *et al.* 2010). Indeed, Lardon *et al.* (2009) estimated that the necessary energy consumption for drying was near 85% of the total energy consumption in a biodiesel production process from microalgae. Another drawback of biodiesel process is associated with the microalgae cultivation step, as nutrient requirements are 55-111 times higher than for e.g. rapeseed cultivation (Halleux *et al.* 2008). Under these conditions, biodiesel production from microalgae may not be energetically and environmentally sustainable (Sialve *et al.* 2009; Ras *et al.* 2011).

2. Microalgae as a source of biogas

Biogas production through anaerobic digestion is an established technology where a wide variety of residues can be used as substrate. In 2011, 8,760 anaerobic digesters were reported in Europe (IEA, 2011). The contribution of this technology to the reduction of carbon emissions, green energy and green gas policies has generated intense interest, especially over the past decade.

When considering biogas production from microalgae two alternatives can be conceived: Microalgae biodiesel production and further anaerobic digestion of microalgae residues for biogas production (Process 1, Figure 1A) and anaerobic digestion of whole microalgae with biogas as sole biofuel (Process 2,Figure 1B).

Process 1: Biodiesel production and subsequent biogas production from spent microalgae. Two principal drawbacks are identified when biodiesel production from microalgae is considered: high nutrients requirements for microalgae growth and low energy efficiency of biodiesel production process. Anaerobic digestion may contribute to overcome such limitations, by enabling nutrients recovery and biogas production when spent microalgae after lipid extraction is used as substrate. This is based on the fact that biogas can be used as source of renewable energy and that during anaerobic digestion process, nitrogen and phosphorus may be recovered, creating opportunities for their reuse as nutrients. Theoretical energy contribution of anaerobic digestion is presented in Figure 1A, assuming microalgae content of lipids, proteins and carbohydrates to be 30, 45 and 25%, respectively.

Figure 1A shows that an energy yield of 11MJ per kilogram of gross microalgae is reached when biodiesel production is considered. If oil extracted microalgae is used as substrate in anaerobic digestion process, methane produced would have a maximum theoretical contribution of 17MJ per kilogram of gross microalgae (thermal). Such value has been computed

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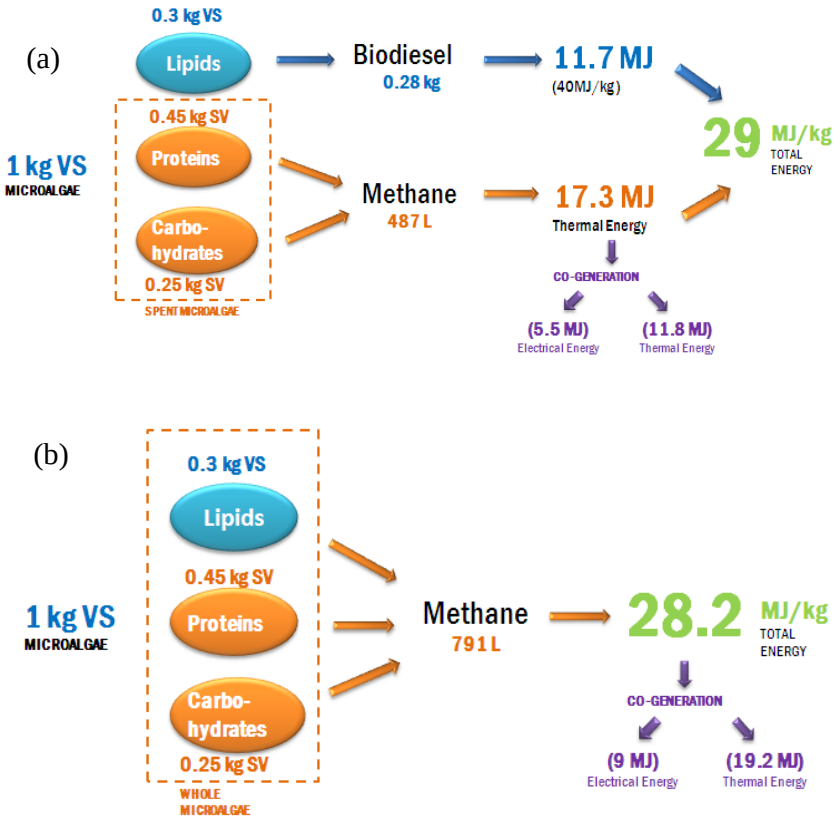

Figure 1. Energy potential of microalgae considering: a) Biodiesel production and further anaerobic digestion of microalgae residues for biogas production or b) Anaerobic digestion of whole microalgae only for biogas production.

assuming carbohydrate and protein methanogenic potentials of 0.415 and 0.851 L CH_4/kg VS, respectively (Angelidaki & Sanders 2004). If the latter thermal energy is transformed into electricity, a maximum energy yield of 5.5 MJ per kilogram of gross microalgae would be achieved (assuming a conversion efficiency of 32%). Thus, a substantial increase in energy yield could be theoretically achieved, representing a considerable contribution to biodiesel sustainability and economic feasibility. Energy contained in biogas can be used for both anaerobic digestion and trans-esterification reactor heating. Electricity obtained via co-generation can be used for different purposes such as photobioreactor mixing, microalgae harvesting and drying (Harun *et al.* 2010; Razon & Tan 2011). Neumann et al. (2011) evaluated energy contribution of biogas production in Process 1 for *Botryococcus braunii* with 30% lipid content. The latter study considered a nutrient recovery step through membrane liquid/solid separation from anaerobic digestion reactor and heptane evaporating step in order to recovery this solvent. Biogas production could theoretically contribute with close to 50% of the overall energy yield of Process 1.

Process 2: Biogas production from whole microalgae. Another alternative to recover energy from microalgae consists of methane production from whole microalgae. In such process, all organic matter (proteins, carbohydrates and lipids) present in microalgae biomass would be converted into methane and carbon dioxide, without considering biodiesel production (De Schamphelaire & Verstraete 2009; Douskova *et al.* 2010; Zamalloa *et al.* 2011). Several advantages are recognized when energy production from whole microalgae through biogas generation is considered: Biogas productions involves high energy yields, biogas production would not require microalgae biomass drying (it involves wet fermentation), biogas can be used to produce heat and electricity through co-generation, microalgae cultures can be used for biogas upgrading (i.e. CO_2 biosequestration), microalgae species not capable of accumulating lipids may be also used as feedstock. Moreover, co-digestion with other types of biomass such as solid or liquid wastes is feasible. Anaerobic digestion of algal and microalgae biomass has been previously studied by some researches (Vergara-Fernández *et al.* 2008; De Schamphelaire & Verstraete 2009; Mussgnug *et al.* 2010; Zamalloa *et al.* 2011). Figure 1B shows the energy potential of Process 2, in which whole microalgae is used as substrate in order to produce biogas. In this estimation, all energy is produced as methane, which allows theoretical maximum energy recovery of 27 MJ per kg of volatile solids of microalgae (8.6MJ of electricity and 18.4 MJ of heat, if co-generation is considered). The lower operational energy demands for biogas production, compared with biodiesel together with biogas, makes Process 2 very promising for energy recovery.

3. Anaerobic digestion of microalgae

Reports of the anaerobic digestion of microalgae go back to the fifties when Golueke *et al*. (1957) was one of the first authors studying the feasibility of sunlight energy conversion to methane by algae sunlight fixation followed by biomass anaerobic fermentation. In this early study, 0.5 m^3 of biogas was obtained per volatile kg of algal biomass, with methane content 63%. More than two decades later, Nair *et al.* (1983) reported a lower yield, close to 0.22 m^3/kg VSS, at loading rate 1.7 kg/(m^3 d). Despite those early reports, biogas production from algae and microalgae has not yet widely researched (Foree & McCarty 1970; Samson & Leduy 1983; Tarwadi & Chauhan 1987; Vergara-Fernández *et al.* 2008; De Schamphelaire & Verstraete 2009; Mussgnug *et al.* 2010; Zamalloa *et al.* 2011).

3.1. Choosing microalgal culture for direct biogas production

The ideal microalgae specie for a maximum biogas production would that presenting:

1. thin or no cell wall
2. large cells
3. high growth rate in non-sterile media
4. high resistivity against natural contaminants

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5. carbohydrate-based cell wall.

Of the above mentioned factors, the quality of cell wall is crucial for anaerobic digestion of algae. This is because cell walls are hard to degrade biologically and their presence avoids contact of anaerobic bacteria with the readily degradable content of algal cells. Therefore, the influence of cell wall presence is described in detail in the following text.

3.1.1. Composition of algal cell wall

Cell wall in microalgae represents 12-36% of total cell mass (cell wall weight/cell weight) in different microalgae (Table 1). Microalgae cell wall is composed mainly of carbohydrates and proteins which represent 30-75% and 1-37% of cell wall, respectively.

Microalgae	Cell Wall	Cell Wall composition (%)			References
	(% w/w)	Carbohydrates	Protein	n.d.*	
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)
Kirchneriella 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 & 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 obliquos	-	39.00	15.00	46.00	(Blumreisinger *et al.* 1983)

* not determined.

Table 1. Cell wall composition of microalgae.

Other compounds found in microalgal cell wall are uronic acid, glucosamine, hidroxyproline, proline, sporopollenin, carotenoids and another resistant biopolymers (Punnett & Derrenbacker 1966; Domozych *et al.* 1980; Blumreisinger *et al.* 1983; Brown 1991, 1992; Abo-Shady *et al.* 1993).

In relation to carbohydrates in microalgae cell wall, neutral sugars, cellulose and hemicelluloses are the main components. Blumreisinger *et al.* (1983) studied five different microalgae in

relation to carbohydrate composition in cell wall, obtaining a prominent neutral sugar component. Composition of cellulose and hemicelluloses has ranged between 6-17% and 18-32% for microalgae studied in other researches carried out by Abo-Shady *et al.* (1993) and Domozych *et al.* (1980), respectively. On the other hand, Northcote *et al.* (1958) reported contents of cellulose near to 45% in cell wall of *Chlorella pirenoidosa*. Unlike these researches, Loos and Meindl (1982) found no presence of cellulose in cell wall of *Clhorella fusca.* In relation to proteins, peptides, proline and hidroxyproline are the main components. According to Punnett and Derrenbacker (1966), the cell wall of six different microalgae consisted of peptides (simple amino acid composition) but it contained no protein. In addition, this research revealed the existence of proline in the cell wall of *Chlorella vulgaris* and hidroxyproline in the cell wall of *Chlorella pyrenoidosa* and *Scenedesmus obliquos*.

3.1.2. Degradability of algal cell wall

Although methane yield is dependent on microalgae composition (Sialve *et al.* 2009), the resistance of cell wall is considered to be the limiting factor for the anaerobic digestion of microalgae (Afi *et al.* 1996; Chen & Oswald 1998). The kinetics of anaerobic digestion is highly dependent on the degradability of the given microalgae species (Sialve *et al.* 2009). Mussgnug *et al.* (2010) studied the methane production from six different microalgae, obtaining from 287 to 587 mL CH_4/ g VS. The low levels of methane yield were related to low cell degradation and high amount of indigestible residues. According to these results, easily degradable microalgae had no cell wall or a protein-based cell wall not containing cellulose/hemicellulose. Batch tests with low methane yields, intact cell walls of microalgae were found with light microscopy in this study. Thus, the intracellular content was not available for efficient digestion. The presence of biopolymers resistant to anaerobic degradation has been reported in the outer cell wall of microalgae species such as *Botryococcus braunii* (Templier *et al.* 1992; Banerjee *et al.* 2002). Moreover, microalgae degradability is related to cell wall structures containing these resistant biopolymers. Some microalgae have a protective tri laminar outer wall called tri laminar sheath (*TLS*), which hinders efficient microalgae degradation (Derenne *et al.* 1992). Thus, higher *TLS* resistance to degradation reported by Derenne *et al.* (1992) for microalgae *B. braunii* has been associated to the presence of sporopollenin-like biopolymers (Kadouri *et al.* 1988; Derenne *et al.* 1992). Other indigestible compound found in microalgae cell wall is algaenan, which has been reported as non-hydrolysable resistant biopolymer composed of polyether linked long-chain (up to C36) n-alkyl units (Gelin *et al.* 1997; Blokker *et al.* 1998; Gelin *et al.* 1999; Simpson *et al.* 2003).

3.1.3. Source of methane in algae

Many authors have related methane yield from microalgae to their composition (Sialve *et al.* 2009; Mairet *et al.* 2011; González-Fernández *et al.* 2012; Mairet *et al.* 2012), especially with the content of lipids, carbohydrates and proteins. However, the experimental data collected from literature do not show strong correlation between lipids, carbohydrates and proteins found in various algal species and the methane yield obtained by various authors (Fig. 2).

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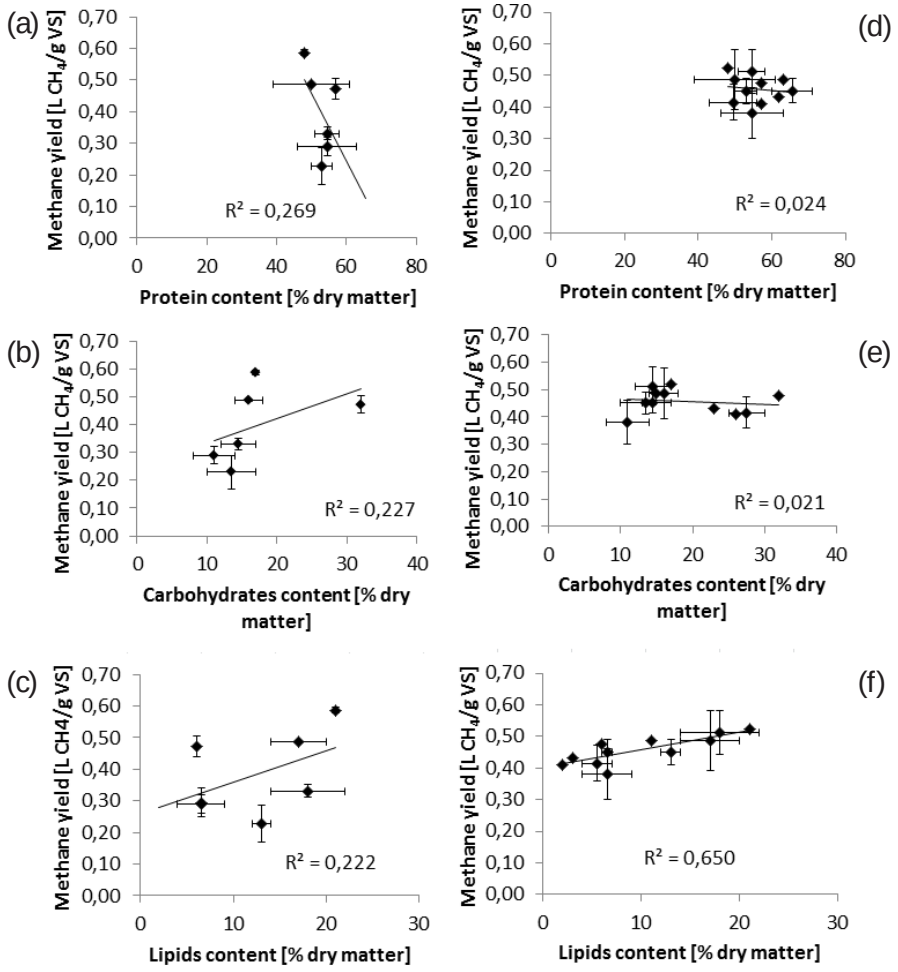

Figure 2. Dependence between methane yield from microalgae and their lipids, carbohydrates and proteins content. Each data point represents one algae species while the error bars show the range found in the literature. Figures (a), (b) and (c) show experimentally obtained methane yields, figures (d), (e) and (f) represent theoretical methane yield for the given algae composition calculated according to Angelidaki and Sanders (2004). Data were extracted from multiple authors (Becker 2007; Griffiths & Harrison 2009; Sialve *et al.* 2009; Mairet *et al.* 2011; González-Fernández *et al.* 2012; Mairet *et al.* 2012).

Angelidaki and Sanders (2004) presented theoretical methane yields from proteins, carbohydrates and lipids of 0.50, 0.42 and 1.01 L/g VS, respectively (Fig. 3). Even when these values are used for calculation of the potential methane yield from various algal species, no strong correlation can be found (Fig. 2d, e and f). Theoretically, lipids content has the biggest influence on methane yield, but as lipids are usually not the mayor source of methane (Fig. 2), the correlation between lipids content and methane yield is still rather vague (Fig. 2).

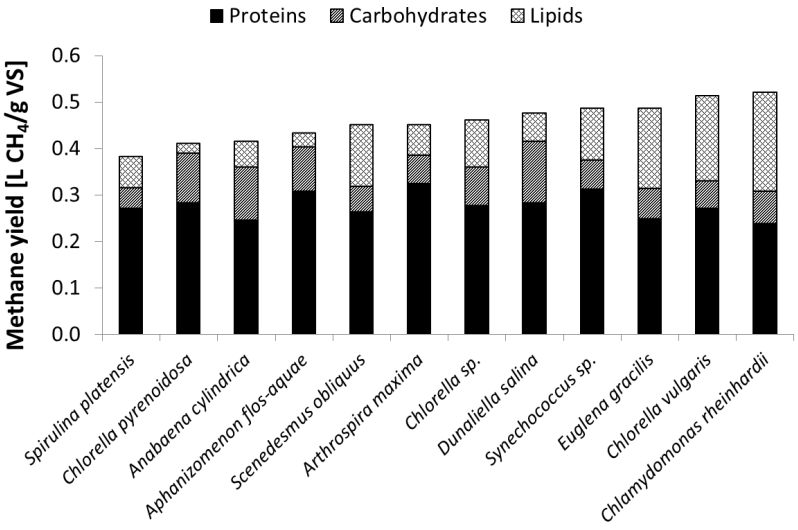

Figure 3. Potential methane yield from proteins, carbohydrates and lipids present in various algae species calculated according to Angelidaki and Sanders (2004). The data on proteins, carbohydrates and lipids content in algae were extracted from Becke (2007), Sialve (2009), Griffiths and Harrison (2009) and González-Fernández et al. (2012).

These facts clearly show that the ration between various macromolecules is not the most important parameter determining the actual methane yield from algae. As it was mentioned before, content of inert organic matter (e.g. cell wall) would play more important role (González-Fernández *et al.* 2012).

These findings show that plain composition of algal biomass indeed cannot be the main factor while choosing the best algal strain for methane production. Biomass production rate and the content of cell-walls will be of higher importance. Moreover, environmental conditions such as the salinity of available water source must be taken into account.

3.2. Pretreatment

In order to overcome limitation caused by cell wall degradability, which is necessary to access the intracellular content, cell disruption (pretreatment) has been pointed out as an important contributor in order to enhance anaerobic digestion efficiency. As mentioned above, cell wall degradability affects both Processes 1 and 2. However, in Process 1, cell wall degradability should not be as critical as in Process 2 since lipid extraction itself may be considered a pretreatment step.

There are different pretreatment techniques applied to microalgae, which can be classified as enzymatic, chemical and mechanical treatments. Mechanical pretreatment include autoclaving, homogenizers, microwaves and sonication, which increases the availability of organic matter (Angelidaki & Ahring 2000). Chemical pretreatment will increase availabil-

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ity of compounds resistant to anaerobic hydrolysis due to the enhanced disintegration (Bonmatí *et al.* 2001).

Chemical pretreatment can be clasified as acid or alkaline treatment. An increase in soluble hemicellulose present in cell wall is expected when alkaline pre-treatment is used (Abo-Shady *et al.* 1993). Thus, chemical pre-treatment is suitable when microalgae cell wall is rich on hemicelluloses. Also, enzymatic pretreatment has been used in order to attack cell wall and improve compounds extraction from microalgae. Enzymatic pretreatment with α-amilase, amylo-glucosidase and cellulase have shown a positive effect on cell wall hydrolysis (Choi *et al.* 2010; Fu *et al.* 2010). Fu *et al.* (2010) reported a 62% increase in cell wall hydrolysis, when *Chlorella sp.* was pretreated by immobilized cellulase.

Few studies report the effect of cell disruption pretreatment in anaerobic digestion (Samson & Leduy 1983; Chen & Oswald 1998). Samson and Leduy (1983) reported an increase of 78% in soluble COD when algae *Spirulina maxima* was mechanically pretreated (sonication and mechanical disintegration). However, no increase in methane yield was observed.

Finally, two considerations should be taken into account when cell disruption pretreatment is evaluated in the context of anaerobic digestion: On one hand, energy consumption associated with pretreatment should be low in order to avoid a negative contribution to the energy balance of anaerobic digestion process. On the other hand, contribution to the biodegradability of the given substrate should be a response variable when the effect of pretreatment on anaerobic digestion is evaluated. In other words, some pretreatment techniques increase solubility of organic matter but do not increase its biodegradability.

3.3. Inhibiting factors related to anaerobic digestion

Figure 1B shows the energy potential when microalgae are used as substrate in order to produce biogas. In this estimation, total energy is produced as methane, which allows a theoretical maximum energy recovery of 27MJ per kg of volatile solids of microalgae. As in Process 1, part of energy produced will be spent for supplying the energy necessary for microalgae harvesting and up-concentration, photobioreactor mixing, photobioreactor and anaerobic reactor heating, etc. The theoretical estimations of energy production from anaerobic digestion presented in this review have been so far computed considering 100% of microalgae biodegradability and high performance of anaerobic digestion. However, an energy production lower than ideal can be expected when limiting factors in anaerobic digestion process are considered. For this reason, this book chapter examines different limiting factors of anaerobic digestion, which are necessary to overcome in order to improve performance of this process.

3.3.1. Ammonium inhibition

Ammonium is presented as protonated form (NH_4^+) and deprotonated form (NH_3, ammonia). The latter is considered to be the specie responsible for the inhibition of anaerobic digestion, due to its permeability through cell membrane (De Baere *et al.* 1984). There are several mechanisms by which ammonia will act as inhibitor of anaerobic bacteria among which are

intracellular pH changes, increase in energy requirements for maintenance and inhibition of specific enzymes (Wittmann *et al.* 1995).

Several factors determining ammonia concentration in anaerobic reactor has been reported, but substrate concentration is a major one (Sialve *et al.* 2009). Distribution of total ammonia between protonated and deprotonated forms strongly depends on factors such as pH and temperature. At high pH values ammonium gets deprotonated forming toxic ammonia (NH_3) (Borja *et al.* 1996). Its inhibitory effect can result in volatile fatty acids accumulation due to a decrease in methanogenic activity, which generates a decrease in pH and ammonia concentration (Chen *et al.* 2008). This interaction may generate an inhibited steady-state, in which the process remains stable despite inhibition (Angelidaki & Ahring 1993; Angelidaki *et al.* 1993). Temperature is another variable that determine NH_4^+/NH_3 ratio, which is directly related to the increase of ammonia fraction and thus, inhibition level (Braun *et al.* 1981; Angelidaki & Ahring 1994).

Microalgal biomass can be expected to have low C/N ratio due to the high protein content in microalgae (Becker 2007). Then, anaerobic degradation of these residues is expected to generate a high ammonium concentration that may cause inhibition of anaerobic microbial consortia, especially methanogenic bacteria (Angelidaki & Ahring 1993; Chen *et al.* 2008). In addition, high ammonium concentration may affect biogas quality since ammonia can be stripped into gas phase (Sialve *et al.* 2009).

During anaerobic digestion of oil extracted microalgae (Process 2 on Figure 1), ammonia inhibition is expected to be especially of concern, since oil extraction will decrease C/N ratio. Figure 4 shows an estimation of the effect of substrate concentration and free ammonia levels in a hypothetical anaerobic digestion reactor. Estimation was calculated considering protein content reported by Becker (2007), operation pH value 8, temperature 35º C, ammonia conversion 90% and total lipid extraction efficiency. Figure 4 shows that inhibitory ammonia concentrations will develop whenever solids concentration exceeding 2% are applied during the anaerobic digestion step. This result was evaluated considering free ammonia inhibition at 100 mg/L NH_3 (dotted line in Figure 4).

Results shown in Figure 4 indicate that that either anaerobic digestion has to be performed at very low levels of solids concentration, or mechanisms for ammonia removal must be implemented. It has to be remained that Figure 4 assumes 90% of conversion of proteins. Lower protein conversions will reduce the chances of ammonia inhibition. However it is clear that this phenomena needs to be addressed if high rate digestion of microalgae is of interest.

One way to overcome this drawback is the possibility of co-digestion in order to provide an optimal C/N ratio for anaerobic digestion process (Yen & Brune 2007; Ehimen *et al.* 2011). Thus, a higher C/N ratio co-substrate should be mixed with microalgae in order to increase anaerobic digestion yield. This strategy is more attractive considering the fact that some co-substrate can stimulate enzymatic synthesis and, hence, increase hydrolysis and degradability (Yen & Brune 2007). Also, co-digestion can dilute toxic compounds decreasing their concentration below toxic/inhibition levels (Sialve *et al.* 2009).

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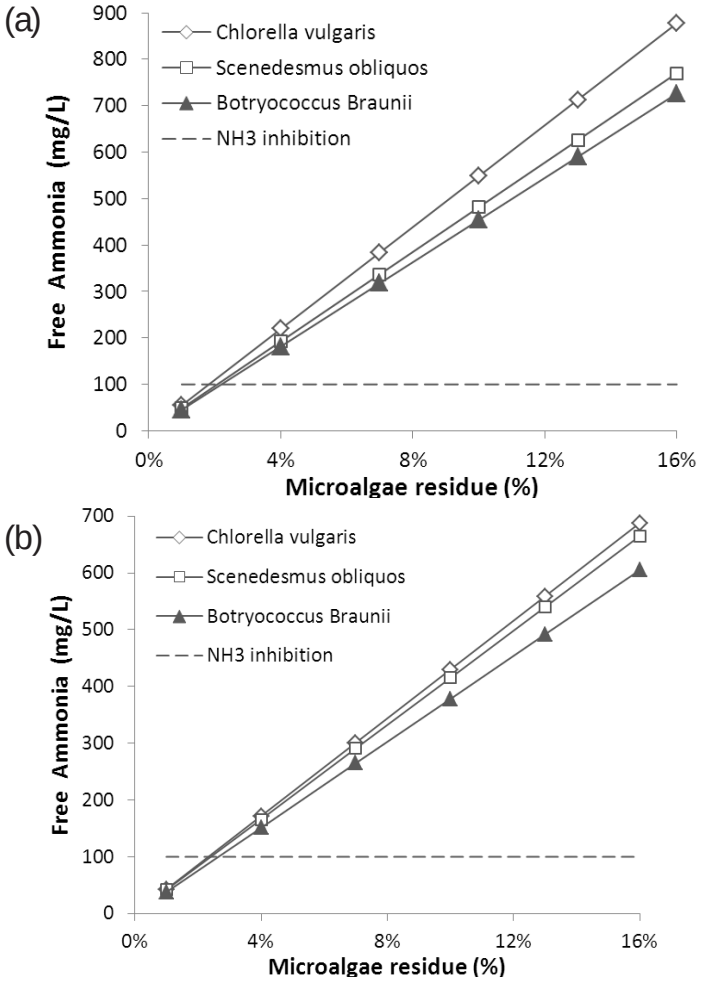

Figure 4. Estimation of free ammonia concentration on anaerobic digestion reactor from substrate level of feedstock, considering (a) processes 1, Biodiesel production and subsequent biogas production from spent microalgae and (b) process 2, Biogas production from whole microalgae.

3.3.2. Salt inhibition

Salt inhibition is expected to be relevant when saline microalgae are used as substrate for biogas production. In those locations where freshwater is not abundant or available, saline microalgae may be of interest, if cultivation takes place close to the sea. In those situations, salinity may even be higher than sea water when open pounds are used, as a result of water evaporation. If biomass is not diluted with fresh water after harvesting, downstream processes such as anaerobic digestion may need to deal with the salinity present in the biomass.

At low concentrations, sodium is essential for methanogenic bacteria. Probably, it is due to its role in ATP formation or NADH oxidation (Dimroth & Thomer 1989). Sodium concentration ranges 100-350mg/L have been reported as beneficial for mesophilic methanogenic growth (McCarty 1965; Patel & Roth 1977). Although moderate concentrations can stimulate bacteria growth, excessive amounts of salt reduce growth rate, and can cause severe inhibition or toxicity (Soto *et al.* 1991). Moreover, high salt levels can cause dehydration in bacteria due to osmotic pressure (De Baere *et al.* 1984; Yerkes *et al.* 1997).

Different levels of saline tolerance in anaerobic bacteria have been reported (Lefebvre & Moletta 2006). Easily degradable substrates seem to increase salt tolerance, most likely as a result of higher energy availability to cope with the energetic requirements of salt tolerance mechanisms (Xiao & Roberts 2010). Rinzema *et al*. (1988) found non acetoclastic methanogenic activity at 16 g/L of sodium concentration. The concentration that generated 50% of activity reduction (IC50) was 10 g/L and no bacteria adaptation after 12 weeks was observed. Similar saline tolerance was observed by Liu and Boone (1991). Feijoo *et al*. (1995) analyzed sodium inhibition for anaerobic bacteria from different reactors. A high tolerance in anaerobic bacteria from reactor treating wastewater under salinity conditions was observed, which was interpreted as consequence of bacteria adaptation. IC50 value for these bacteria was 16.3 g Na^+/L and entire inhibition was observed at 21 g Na^+/L.

Several reports indicate that biomass acclimation may significantly increase the activity under saline conditions (Soto *et al.* 1991; Omil *et al.* 1995; Chen *et al.* 2008; Kimata-Kino *et al.* 2011). However, reports are also available where no or little acclimating was observed (Aspe *et al.* 1997). Then, selection rather than adaptation is likely to be the mechanisms to provide high activity when big changes in salinity are imposed, requiring the presence of salinity-tolerant microorganisms in the inoculum (Gebauer 2004). It is indeed a common practice to use inoculums containing sources of saline resistant microorganisms, such as marine sediments (Xiao & Roberts 2010).

3.4. Biogas upgrading

Many biogas applications such as vehicle use, household distribution and electricity production, require some level of biogas upgrading to remove impurities or to increase methane content.

CO_2 removal is a key factor in order to obtain a higher calorific value of biogas. Processes such as solvent absorption, activated carbon adsorption and membrane filtration have been used for CO_2 removal (Kapdi *et al.* 2005; Makaruk *et al.* 2010; Ryckebosch *et al.* 2011).

Photosynthetic microorganisms such microalgae can also be used to remove CO_2 from biogas. Microalgae cultures are regarded as an interesting tool for carbon dioxide capture from gases such as flue gases from boilers, combustion engines or thermal power plants. This would not only alleviate impact of CO_2 emissions on the environment, but it would also reduce the cost of microalgae production (Doucha *et al.* 2005; Ryu *et al.* 2009). Stabilization ponds have been already recognized as potential CO_2 scrubbers due to their (micro-) algae growth (Shilton *et al.* 2008). Several authors have reported the successful

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growth of microalgae using flue gases. Negoro *et al.* (1993) reported productivities similar to those using pure CO_2, and showed that growth was barely influenced by the content of SO_X and NO_X contained in flue gases. Similar results were obtained by Hauck *et al.* (1996) who found no inhibition of *Chlorella sp.* by the levels of NO_X typically contained in flue gases. Doucha *et al.* (2005) reported 50% of flue gas decarbonization when working with a photobioreactor. In this study, 4.4 kg of CO_2 was needed for the production of 1 kg of dried algal biomass.

Conde *et al.* (1993) achieved biogas purification in laboratory experiments up to methane content of 97% with algae grown on synthetic nutrient medium. Mandeno *et al.* (2005) achieved CO_2 reduction from 40 to less than 5% using synthetic biogas, observing little transfer of oxygen to the biogas, so explosive methane/oxygen mixtures would not be formed. Similar results in terms of CO_2 reduction were obtained by Travieso *et al*. (1993) working with real biogas. Several microalgae species such as *Chlorococcum littorale*, *Chlorella sp*., *Chlorella sp*. UK001, *Chlorella vulgaris*, *Chlorella kessleri*, *Scenedesmus obliquus, Spirulina sp., Haematococcus pluvialis* or *Botryococcus braunii* have shown high levels of tolerance to high partial pressures of CO_2 (Wang *et al.* 2008; Brennan & Owende 2010). Mass transfer of carbon dioxide from gas to liquid phase is dependent on several factors highlighting chemical balance in microalgae media, pH and flow pattern of reactor in which culture is growing (Kumar *et al.* 2010). However, no full scale installations are under operation with this concept.

Available publications do not report negative effects of high methane partial pressures over microalgae cultures. Moreover, Meier *et al*. (2011) reported no inhibition effect when exposing a culture of *N. gaditana* to atmospheres containing methane up to 100%.

Hydrogen sulfide is present in biogas at low concentrations although its treatment should be considered. Some studies have reported a hydrogen sulphide decrease after biogas is upgraded in microalgae culture (Conde *et al.* 1993; Heubeck *et al.* 2007; Sialve *et al.* 2009). Most likely, hydrogen sulphide removal should be attributed to relative high solubility in growth medium (Conde *et al.* 1993; Sialve *et al.* 2009). Solubilised hydrogen sulphide can be easily oxidized into sulphate due to oxygen presence in growth medium.

4. Conclusions

Microalgal biomass is a promising substrate for renewable energy production. In this book chapter, direct anaerobic digestion without previous biodiesel extraction was shown to be the most promising method of energy production from microalgae. Lipids used for biodiesel production can also serve as a rich source of biogas with energetic efficiency higher than when microalgae are used for subsequent biodiesel and biogas production. The higher energy efficiency is given mostly by the simple technology with low energy demand used for methane production. These benefits combined with the possibility of CO_2 and nutrients recycling from the anaerobic effluents make anaerobic digestion the best technology for removable energy production from microalgae.

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References

[1] Abo-Shady A. M., Mohamed Y. A. and Lasheen T. (1993). Chemical composition of the cell wall in some green algae species. Biologia Plantarum 35(4), 629-32, ISSN: 1573-8264

[2] Afi L., Metzger P., Largeau C., Connan J., Berkaloff C. and Rousseau B. (1996). Bacterial degradation of green microalgae: Incubation of Chlorella emersonii and Chlorella vulgaris with Pseudomonas oleovorans and Flavobacterium aquatile. Organic Geochemistry 25(1-2), 117-30, ISSN: 0146-6380

[3] Amin S. (2009). Review on biofuel oil and gas production processes from microalgae. Energy Conversion and Management 50(7), 1834-40, ISSN: 0196-8904

[4] Angelidaki I. and Ahring B. K. (1993). Thermophilic anaerobic digestion of livestock waste: The effect of ammonia. Applied Microbiology and Biotechnology 38(4), 560-4, ISSN: 1432-0614

[5] Angelidaki I. and Ahring B. K. (1994). Anaerobic thermophilic digestion of manure at different ammonia loads: Effect of temperature. Water Research 28(3), 727-31, ISSN: 0043-1354

[6] Angelidaki I. and Ahring B. K. (2000). Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. Water Science and Technology 41 (3), 189-94, ISSN: 0273-1223

[7] Angelidaki I., Ellegaard L. and Ahring B. K. (1993). A mathemathical model for dynamic simulation of anaerobic digestion of complex substrates, focusing on ammonia inhibition. Biotechnology and Bioengineering 42, 159-66, ISSN: 1097-0290

[8] Angelidaki I. and Sanders W. (2004). Assessment of the anaerobic biodegradability of macropollutants. Reviews in Environmental Science and Biotechnology 3(2), 117-29, ISSN: 1569-1705

[9] Aspe E., Marti M. C. and Roeckel M. (1997). Anaerobic treatment of fishery wastewater using a marine sediment inoculum. Water Research 31(9), 2147-60 ISSN: 0043-1354

[10] Banerjee A., Sharma R., Chisti Y. and Banerjee U. C. (2002). Botryococcus braunii: A renewable source of hydrocarbons and other chemicals. Critical Reviews in Biotechnology 22(3), 245-79, ISSN: 1549-7801

[11] Becker E. W. (2007). Micro-algae as a source of protein. Biotechnology Advances 25(2), 207-10, ISSN: 0734-9750

[12] Blokker P., Schouten S., Van den Ende H., De Leeuw J. W., Hatcher P. G. and Sinninghe Damsté J. S. (1998). Chemical structure of algaenans from the fresh water algae Tetraedron minimum, Scenedesmus communis and Pediastrum boryanum. Organic Geochemistry 29(5-7 -7 pt 2), 1453-68, ISSN: 0146-6380

[13] Blumreisinger M., Meindl D. and Loos E. (1983). Cell wall composition of chlorococcal algae. Phytochemistry 22(7), 1603-4, ISSN: 0031-9422

[14] Bonmatí A., Flotats X., Mateu L. and Campos E. (2001). Study of thermal hydrolysis as a pretreatment to mesophilic anaerobic digestion of pig slurry. Water Science and Technology 44 (4), 109-16, ISSN: 0273-1223

[15] Borja R., Sánchez E. and Durán M. M. (1996). Effect of the clay mineral zeolite on ammonia inhibition of anaerobic thermophilic reactors treating cattle manure. Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering 31(2), 479-500, ISSN: 1532-4117

[16] Braun R., Huber P. and Meyrath J. (1981). Ammonia toxicity in liquid piggery manure digestion. Biotechnology Letters 3(4), 159-64, ISSN: 1573-6776

[17] Brennan L. and Owende P. (2010). Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. Renewable and Sustainable Energy Reviews 14(2), 557-77, ISSN: 1364-0321

[18] Brown M. R. (1991). The amino-acid and sugar composition of 16 species of microalgae used in mariculture. Journal of Experimental Marine Biology and Ecology 145(1), 79-99, ISSN: 0022-0981

[19] Brown M. R. (1992). Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae. 1. Amino acids Sugars and pigments. Journal of Experimental Marine Biology and Ecology 161(1), 91-113, ISSN: 0022-0981

[20] Conde J. L., Moro L. E., Travieso L., Sanchez E. P., Leiva A., Dupeiron R. and Escobedo R. (1993). Biogas purification process using intensive microalgae cultures. Biotechnology Letters 15(3), 317-20, ISSN: 1573-6776

[21] Costa J. A. V. and de Morais M. G. (2011). The role of biochemical engineering in the production of biofuels from microalgae. Bioresource Technology 102(1), 2-9, ISSN: 0960-8524

[22] Chen P. H. and Oswald W. J. (1998). Thermochemical treatment for algal fermentation. Environment International 24(8), 889-97, ISSN: 0160-4120

[23] Chen Y., Cheng J. J. and Creamer K. S. (2008). Inhibition of anaerobic digestion process: A review. Bioresource Technology 99(10), 4044-64, ISSN: 0960-8524

[24] Chisti Y. (2007). Biodiesel from microalgae. Biotechnology Advances 25(3), 294-306, ISSN: 0734-9750

[25] Choi S. P., Nguyen M. T. and Sim S. J. (2010). Enzymatic pretreatment of Chlamydomonas reinhardtii biomass for ethanol production. Bioresource Technology 101(14), 5330-6, ISSN: 0960-8524

[26] De Baere L. A., Devocht M., Van Assche P. and Verstraete W. (1984). Influence of high NaCl and NH4Cl salt levels on methanogenic associations. Water Research 18(5), 543-8, ISSN: 0043-1354

[27] De Schamphelaire L. and Verstraete W. (2009). Revival of the biological sunlight-to-biogas energy conversion system. Biotechnology and Bioengineering 103(2), 296-304, ISSN: 1097-0290

[28] Derenne S., Largeau C., Berkaloff C., Rousseau B., Wilhelm C. and Hatcher P. G. (1992). Non-hydrolysable macromolecular constituents from outer walls of Chlorella fusca and Nanochlorum eucaryotum. Phytochemistry 31(6), 1923-9, ISSN: 0031-9422

[29] Dimroth P. and Thomer A. (1989). A primary respiratory Na+ pump of an anaerobic bacterium: the Na+-dependent NADH:quinone oxidoreductase of Klebsiella pneumoniae. Archives of Microbiology 151(5), 439-44, ISSN: 1432-072X

[30] Domozych D. S., Stewart K. D. and Mattox K. R. (1980). The comparative aspects of cell wall chemistry in the green algae (Chlorophyta). Journal of Molecular Evolution 15(1), 1-12, ISSN: 1432-1432

[31] Doucha J., Straka F. and Lívanský K. (2005). Utilization of flue gas for cultivation of microalgae (Chlorella sp.) in an outdoor open thin-layer photobioreactor. Journal of Applied Phycology 17(5), 403-12, ISSN: 1573-5176

[32] Douskova I., Kastanek F., Maleterova Y., Kastanek P., Doucha J. and Zachleder V. (2010). Utilization of distillery stillage for energy generation and concurrent production of valuable microalgal biomass in the sequence: Biogas-cogeneration-microalgae-products. Energy Conversion and Management 51(3), 606-11, ISSN: 0196-8904

[33] Ehimen E. A., Sun Z. F., Carrington C. G., Birch E. J. and Eaton-Rye J. J. (2011). Anaerobic digestion of microalgae residues resulting from the biodiesel production process. Applied Energy 88(10), 3454-63, ISSN: 0306-2619

[34] Feijoo G., Soto M., Méndez R. and Lema J. M. (1995). Sodium inhibition in the anaerobic digestion process: Antagonism and adaptation phenomena. Enzyme and Microbial Technology 17(2), 180-8, ISSN: 0141-0229

[35] Foree E. G. and McCarty P. L. (1970). Anaerobic decomposition of algae. Environmental Science and Technology 4(10), 842-9, ISSN: 1520-5851

[36] Fu C. C., Hung T. C., Chen J. Y., Su C. H. and Wu W. T. (2010). Hydrolysis of microalgae cell walls for production of reducing sugar and lipid extraction. Bioresource Technology 101(22), 8750-4, ISSN: 0960-8524

[37] Gebauer R. (2004). Mesophilic anaerobic treatment of sludge from saline fish farm effluents with biogas production. Bioresource Technology 93(2), 155-67, ISSN: 0960-8524

[38] Gelin F., Boogers I., Noordeloos A. A. M., Sinninghe Damsté J. S., Riegman R. and De Leeuw J. W. (1997). Resistant biomacromolecules in marine microalgae of the classes eustigmatophyceae and chlorophyceae: Geochemical implications. Organic Geochemistry 26(11-12), 659-75, ISSN: 0146-6380

[39] Gelin F., Volkman J. K., Largeau C., Derenne S., Sinninghe Damsté J. S. and De Leeuw J. W. (1999). Distribution of aliphatic, nonhydrolyzable biopolymers in marine microalgae. Organic Geochemistry 30(2-3), 147-59, ISSN: 0146-6380

[40] Golueke C. G., Oswald W. J. and Gotaas H. B. (1957). Anaerobic digestion of Algae. Applied microbiology 5(1), 47-55, ISSN: 1365-2672

[41] González-Fernández C., Sialve B., Bernet N. and Steyer J. P. (2012). Impact of microalgae characteristics on their conversion to biofuel. Part II: Focus on biomethane production. Biofuels, Bioproducts and Biorefining 6(2), 205-18, ISSN: 1932-1031

[42] Griffiths M. and Harrison S. (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. Journal of Applied Phycology 21(5), 493-507, ISSN: 0921-8971

[43] Halleux H., Lassaux S., Renzoni R. and Germain A. (2008). Comparative life cycle assessment of two biofuels: Ethanol from sugar beet and rapeseed methyl ester. International Journal of Life Cycle Assessment 13(3), 184-90, ISSN: 1614-7502

[44] Harun R., Davidson M., Doyle M., Gopiraj R., Danquah M. and Forde G. (2010). Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility. Biomass and Bioenergy 35(1), 741-7, ISSN: 0961-9534

[45] Hauck J. T., Olson G. J., Scierka S. J., Perry M. B. and Ataai M. M. (1996). Effects of simulated flue gas on growth of microalgae. ACS Division of Fuel Chemistry, Preprints 41(4), 1391-4

[46] Heubeck S., Craggs R. J. and Shilton A. (2007). Influence of CO2 scrubbing from biogas on the treatment performance of a high rate algal pond. Water Science and Technology 55 (11), 193-200, ISSN: 0273-1223

[47] Kadouri A., Derenne S., Largeau C., Casadevall E. and Berkaloff C. (1988). Resistant biopolymer in the outer walls of Botryococcus braunii, B race. Phytochemistry 27(2), 551-7, ISSN: 0031-9422

[48] Kapdi S. S., Vijay V. K., Rajesh S. K. and Prasad R. (2005). Biogas scrubbing, compression and storage: Perspective and prospectus in Indian context. Renewable Energy 30(8), 1195-202, ISSN: 0960-1481

[49] Kimata-Kino N., Ikeda S., Kurosawa N. and Toda T. (2011). Saline adaptation of granules in mesophilic UASB reactors. International Biodeterioration & Biodegradation 65(1), 65-72, ISSN: 0964-8305

[50] Kumar A., Ergas S., Yuan X., Sahu A., Zhang Q., Dewulf J., Malcata F. X. and van Langenhove H. (2010). Enhanced CO2 fixation and biofuel production via microalgae: Recent developments and future directions. Trends in Biotechnology 28(7), 371-80, ISSN: 0167-7799

[51] Lardon L., Hélias A., Sialve B., Steyer J. P. and Bernard O. (2009). Life-cycle assessment of biodiesel production from microalgae. Environmental Science and Technology 43(17), 6475-81, ISSN: 1520-5851

[52] Lefebvre O. and Moletta R. (2006). Treatment of organic pollution in industrial saline wastewater: A literature review. Water Research 40(20), 3671-82, ISSN: 0043-1354

[53] Liu Y. and Boone D. R. (1991). Effects of salinity on methanogenic decomposition. Bioresource Technology 35(3), 271-3, ISSN: 0960-8524

[54] Loos E. and Meindl D. (1982). Composition of the cell wall of Chlorella fusca. Planta 156(3), 270-3, ISSN: 1432-2048

[55] Mairet F., Bernard O., Cameron E., Ras M., Lardon L., Steyer J. P. and Chachuat B. (2012). Three-reaction model for the anaerobic digestion of microalgae. Biotechnology and Bioengineering 109(2), 415-25, ISSN: 00063592

[56] Mairet F., Bernard O., Ras M., Lardon L. and Steyer J.-P. (2011). Modeling anaerobic digestion of microalgae using ADM1. Bioresource Technology 102(13), 6823-9, ISSN: 0960-8524

[57] Makaruk A., Miltner M. and Harasek M. (2010). Membrane biogas upgrading processes for the production of natural gas substitute. Separation and Purification Technology 74(1), 83-92, ISSN: 1383-5866

[58] Mandeno G., Craggs R., Tanner C., Sukias J. and Webster-Brown J. (2005). Potential biogas scrubbing using a high rate pond. Water Science and Technology 51(12), 253-6, ISSN: 0273-1223

[59] McCarty P. L. (1965). Thermodynamics of biological synthesis and growth. Air and water pollution 9(10), 621-39, ISSN: 0568-3408

[60] Meier L., Torres A., Azocar L., Neumann P., Vergara C., Rivas M. and Jeison D. (2011). Biogas upgrading through microalgae culture: Effect of methane concentration on microalgae activity. In: X Latinamerican and Symposium on Anaerobic Digestion, Ouro Preto, Brazil.

[61] Mussgnug J. H., Klassen V., Schlüter A. and Kruse O. (2010). Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. Journal of Biotechnology 150(1), 51-6, ISSN: 0168-1656

[62] Mutanda T., Ramesh D., Karthikeyan S., Kumari S., Anandraj A. and Bux F. (2011). Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. Bioresource Technology 102(1), 57-70, ISSN: 0960-8524

[63] Nair K. V. K., Kannan V. and Sebastian S. (1983). Bio-gas generation using microalgae and macrophytes. Indian Journal of Environmental Health 24(4), 277-84, ISSN: 0367-827X

[64] Negoro M., Hamasaki A., Ikuta Y., Makita T., Hirayama K. and Suzuki S. (1993). Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. Applied biochemistry and biotechnology 39-40(1), 643-53, ISSN: 1559-0291

[65] Neumann P., Torres A., Azocar L., Meier L., Vergara C. and Jeison D. (2011). Biogas production as a tool for increasing sustainability of biodiesel production from microalgae Botryococcus braunii. In: X Latinamerican and Symposium on Anaerobic Digestion, Ouro Preto, Brazil.

[66] Northcote D. H., Goulding K. J. and Horne R. W. (1958). The chemical composition and structure of the cell wall of Chlorella pyrenoidosa. The Biochemical journal 70(3), 391-7, ISSN: 1470-8728

[67] Omil F., Mendez R. and Lema J. M. (1995). Anaerobic treatment of saline wastewaters under high sulphide and ammonia content. Bioresource Technology 54(3), 269-78, ISSN: 0960-8524

[68] Patel G. B. and Roth L. A. (1977). Effect of sodium chloride on growth and methane production of methanogens. Canadian Journal of Microbiology 23(7), 893-7, ISSN: 1480-3275

[69] Punnett T. and Derrenbacker E. C. (1966). The amino acid composition of algal cell walls. Journal of General Microbiology 44(1), 105-14, ISSN: 0022-1287

[70] Ras M., Lardon L., Bruno S., Bernet N. and Steyer J.-P. (2011). Experimental study on a coupled process of production and anaerobic digestion of Chlorella vulgaris. Bioresource Technology 102(1), 200-6, ISSN: 0960-8524

[71] Razon L. F. and Tan R. R. (2011). Net energy analysis of the production of biodiesel and biogas from the microalgae: Haematococcus pluvialis and Nannochloropsis. Applied Energy 88(10), 3507-14, ISSN: 0306-2619

[72] Rinzema A., Van Lier J. and Lettinga G. (1988). Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor. Enzyme and Microbial Technology 10(1), 24-32, ISSN: 0141-0229

[73] Ryckebosch E., Drouillon M. and Vervaeren H. (2011). Techniques for transformation of biogas to biomethane. Biomass and Bioenergy 35(5), 1633-45, ISSN: 0961-9534

[74] Ryu H. J., Oh K. K. and Kim Y. S. (2009). Optimization of the influential factors for the improvement of CO2 utilization efficiency and CO2 mass transfer rate. Journal of Industrial and Engineering Chemistry 15(4), 471-5, ISSN: 1226-086X

[75] Samson R. and Leduy A. (1983). Influence of mechanical and thermochemical pretreatments on anaerobic digestion of Spirulinamaxima algal biomass. Biotechnology Letters 5(10), 671-6, ISSN: 1573-6776

[76] Scott S. A., Davey M. P., Dennis J. S., Horst I., Howe C. J., Lea-Smith D. J. and Smith A. G. (2010). Biodiesel from algae: Challenges and prospects. Current Opinion in Biotechnology 21(3), 277-86, ISSN: 0958-1669

[77] Shilton A. N., Mara D. D., Craggs R. and Powell N. (2008). Solar-powered aeration and disinfection, anaerobic co-digestion, biological CO2 scrubbing and biofuel production: The energy and carbon management opportunities of waste stabilisation ponds. Water Science and Technology 58 (1), 253-8, ISSN: 0273-1223

[78] Sialve B., Bernet N. and Bernard O. (2009). Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnology Advances 27(4), 409-16, ISSN: 0734-9750

[79] Simpson A. J., Zang X., Kramer R. and Hatcher P. G. (2003). New insights on the structure of algaenan from Botryoccocus braunii race A and its hexane insoluble botryals based on multidimensional NMR spectroscopy and electrospray-mass spectrometry techniques. Phytochemistry 62(5), 783-96, ISSN: 0031-9422

[80] Soto M., Mendez R. and Lema J. M. (1991). Biodegradability and toxicity in the anaerobic treatment of fish canning wastewaters. Environmental Technology 12(8), 669-77, ISSN: 0959-3330

[81] Stephens E., Ross I. L., King Z., Mussgnug J. H., Kruse O., Posten C., Borowitzka M. A. and Hankamer B. (2010). An economic and technical evaluation of microalgal biofuels. Nature Biotechnology 28(2), 126-8, ISSN: 1087-0156

[82] Tarwadi S. J. and Chauhan V. D. (1987). Seaweed biomass as a source of energy. Energy 12(5), 375-8, ISSN: 0360-5442

[83] Templier J., Largeau C., Casadevall E. and Berkaloff C. (1992). Chemical inhibition of resistant biopolymers in outer walls of the A and B races of Botryococcus braunii. Phytochemistry 31(12), 4097-104, ISSN: 0031-9422

[84] Travieso L., Sanchez E. P., Benitez F. and Conde J. L. (1993). Arthrospira sp. intensive cultures for food and biogas purification. Biotechnology Letters 15(10), 1091-4, ISSN: 1573-6776

[85] Vergara-Fernández A., Vargas G., Alarcón N. and Velasco A. (2008). Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. Biomass and Bioenergy 32(4), 338-44, ISSN: 0961-9534

[86] Wang B., Li Y., Wu N. and Lan C. Q. (2008). CO2 bio-mitigation using microalgae. Applied Microbiology and Biotechnology 79(5), 707-18, ISSN: 1432-0614

[87] Wittmann C., Zeng A. P. and Deckwer W. D. (1995). Growth inhibition by ammonia and use of a pH-controlled feeding strategy for the effective cultivation of Mycobacterium chloropheolicum. Applied Microbiology and Biotechnology 44(3-4), 519-25, ISSN: 1432-0614

[88] Xiao Y. Y. and Roberts D. J. (2010). A review of anaerobic treatment of saline wastewater. Environmental Technology 31(8-9), 1025-43, ISSN: 0959-3330

[89] Yen H.-W. and Brune D. E. (2007). Anaerobic co-digestion of algal sludge and waste paper to produce methane. Bioresource Technology 98(1), 130-4, ISSN: 0960-8524

[90] Yerkes D. W., Boonyakitsombut S. and Speece R. E. (1997). Antagonism of sodium toxicity by the compatible solute betaine in anaerobic methanogenic systems. Water Science and Technology 36 (6-7), 15-24, ISSN: 0273-1223

[91] Zamalloa C., Vulsteke E., Albrecht J. and Verstraete W. (2011). The techno-economic potential of renewable energy through the anaerobic digestion of microalgae. Bioresource Technology 102(2), 1149-58, ISSN: 0960-8524