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Research review paper

**Anaerobic digestion of microalgae as a necessary step to make
microalgal biodiesel sustainable**

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Abstract

The potential of microalgae as a source of biofuels and as a technological solution for CO₂ fixation is subject to intense academic and industrial research. In the perspective of setting up massive cultures, the management of large quantities of residual biomass and the high amounts of fertilizers must be considered. Anaerobic digestion is a key process that can solve this waste issue as well as the economical and energetic balance of such a promising technology. Indeed, the conversion of algal biomass after lipid extraction into methane is a process that can recover more energy than the energy from the cell lipids. Three main bottlenecks are identified to digest microalgae. First, the biodegradability of microalgae can be low depending on both the biochemical composition and the nature of the cell wall. Then, the high cellular protein content results in ammonia release which can lead to potential toxicity. Finally, the presence of sodium for marine species can also affect the digester performance. Physico-chemical pretreatment, co-digestion, or control of gross composition are strategies that can significantly and efficiently increase the conversion yield of the algal organic matter into methane. When the cell lipid content does not exceed 40 %, anaerobic digestion of the whole biomass appears to be the optimal strategy on an energy balance basis, for the energetic recovery of cell biomass. Lastly, the ability of these CO₂ consuming microalgae to purify biogas and concentrate methane is discussed.

Keywords: anaerobic digestion, microalgae, biochemical methane potential, codigestion, pretreatment, biogas, CO₂ mitigation, biofuel

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59 1 Introduction

60
61 The potential of photosynthetic microorganisms as an alternative to biofuel crops together
62 with their potential as a promising technology for CO₂ fixation is a subject of strong interest
63 (Chisti, 2007, 2008; Li, 2008)[R3Q5]. Indeed, some eukaryotic microalgae and prokaryotic
64 (cyanobacteria) microorganisms (abusively gathered under the term microalgae in the
65 following) can synthesize lipids under certain environmental conditions (Metting,
66 1996)[R2Q1]. The perspective of large scale production of microalgae for biofuel applications
67 is motivated by the high productivity which can be reached (Huntley and Redalje, 2007;
68 Chisti, 2007; Carlsson et al., 2007). Chisti (2007) considered a scenario where productions up
69 to 127 tons.ha⁻¹.year⁻¹ can be achieved in high rate raceway ponds while Carlsson et al. (2007)
70 suggested an hypothesis of 50 to 60 tons.ha⁻¹.year⁻¹. Photobioreactor productions of up to 150
71 tons.ha⁻¹.year⁻¹ have already been obtained (Carlsson et al., 2007), and Chisti (2007)
72 suggested an upper value of 263 tons.ha⁻¹.year⁻¹.

73 On the basis of an average composition of microalgae given by CO_{0.48}H_{1.83}N_{0.11}P_{0.01}
74 (Grobelaar, 2004) the nitrogen and phosphorus requirement per unit of surface and per year
75 can be estimated. This leads to a nitrogen amendment that varies from 8 to 16 tons N.ha⁻¹
76 .year⁻¹. This figure is in a range 55 to 111 times higher than for rapeseed (Halleux et al.
77 2008)[R1Q1]. This shows that microalgae will involve huge quantities of nitrogen and
78 phosphate for which environmental and economic impact may not be sustainable. A process
79 to recycle nitrogen and phosphorus contained in algal waste after lipid extraction is therefore
80 required in order to reduce the use of fertilizers. Anaerobic digestion can be an answer to this
81 problem, since this biotechnological process can mineralise algal waste containing organic
82 nitrogen and phosphorus, resulting in a flux of ammonium and phosphate that can then be
83 used as a substrate for the microalgae (Olguin, 2000; Phang, 2000). Furthermore, to reach an
84 economical balance, Chisti (2007) has shown that the resulting biomass after lipid extraction
85 needs to be transformed into methane. Thus, if anaerobic digestion is used to process algal
86 waste, it will not only recycle nitrogen and phosphorus but also produce methane. The
87 energetic value[R1Q2] of the produced methane can potentially lead to an energetic balance
88 of the microalgae to biofuel process.

89 Anaerobic degradation of phytoplanktonic cells is a process which takes place naturally
90 in aquatic environments. When algal cells sink towards the anoxic and aphotic zones, they
91 eventually die and break up. Nutrient remineralisation in these anoxic layers of aquatic
92 environments is a key process, responsible for recycling nutritive elements. It leads to
93 ammonium and phosphate release, which can eventually sustain growth of phytoplanktonic
94 communities. This decomposition is a slow and incomplete process. Some cell structures can
95 still be found in the sediments after many years, some being identified in kerogen rocks
96 (Vandenbroucke and Largeau, 2007). Kinetics of these anaerobic degradation processes is
97 highly dependant upon both the species degradability as well as the environmental conditions,
98 eventually resulting in various fractions (Vandenbroucke and Largeau, 2007). The resistance
99 of the cell wall is generally one of the limiting factors for cell digestibility (Chen, 1987; Afi et

100 al. 1996; Chen and Oswald, 1998). Studies of kerogen rocks have shown that remaining
101 fractions of TLS (TriLaminar Sheaths) are correlated to the sedimentation of chlorophyceae
102 (Derenne et al., 1992). These parietal structures characterizing some species enhances
103 resistance to decomposition and intervene in selective safeguarding during the process of
104 fossilization (Vandenbroucke and Largeau, 2007).

105 This natural process has been the subject of research studies since the fifties when energy
106 recovery[R1Q2] of microalgae by anaerobic digestion was investigated. Goeluke and Oswald
107 (1957) published the first study on anaerobic digestion of an algal biomass. In 1960, they
108 proposed an integrated process associating the production of microalgae in an open pond for
109 the treatment of sewage water and the energetic recovery[R1Q2] of the algal biomass by
110 anaerobic digestion (Oswald and Goeluke, 1960). This scientific enthusiasm for methane
111 production as a source of renewable energy awoke again in the nineteen seventies in relation
112 to the first oil crisis.

113 The strong and recent re-interest for anaerobic digestion is correlated to its ability to treat
114 and to convert a wide range of organic wastes into renewable energy. However, in the specific
115 case of microalgal biomass, the available literature is, at present, particularly scarce. Besides
116 the need for the management of algal waste biomass and the energetic interest, the idea of
117 domesticating the microbial loop that takes place in the natural environment (Caron, 1994),
118 and thus the nitrogen and phosphorus recycling is the major issue that we address.

119 [R1Q3]

120 This paper reviews the potential of microalgae as a substrate for anaerobic digestion.
121 First, the characteristics of microalgae regarding their biochemical composition are described
122 and the theoretical methane yield is proposed, based on their gross composition. Secondly,
123 experiments of anaerobic digestion of microalgae and the strategies to improve their
124 conversion into methane are reported.

125

126 **2 Anaerobic digestion of microalgae**

127

128 *2.1 Microalgae composition*

129 Determining the composition of microalgae is a way to apprehend their digestion
130 potential. The mineral composition of microalgae meets the nutrient requirements of the
131 anaerobic microflora. Besides carbon, nitrogen and phosphorus which are major components
132 in microalgae composition, oligo nutrients such as iron, cobalt, zinc are also found
133 (Grobelaar, 2004) and are known to stimulate methanogenesis (Speece, 1996).

134 These organisms have proportions of proteins (6-52 %), lipids (7-23 %) and
135 carbohydrates (5-23%) that are strongly species dependent (Brown et al., 1997). For several
136 species the high proportion in proteins is characterised by a low C/N especially if compared
137 with terrestrial plants. This ratio has an average of 10.2 for freshwater microalgae while it is
138 36 for terrestrial plants (Elser et al. 2000)[R2Q2]. Cell composition is also deeply affected by
139 environmental conditions (Droop, 1983; Leadbeater, 2006). Variations in this composition
140 may affect the performance of the anaerobic digestion.

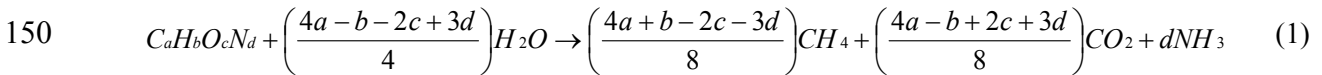
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143 *2.2 Theoretical approach of methane potential and ammonium release*

144 According to Angelidaki and Sanders (2004), when the composition of the organic matter
145 is known, it is possible to evaluate the theoretical methane and ammonium yields that can be
146 expected from the anaerobic digestion. These yields can be calculated with the following

147 formula adapted from Symons and Buswell (1933) . However, it must be kept in mind that
 148 this theoretical approach does not take into account needs for cell maintenance and anabolism.
 149



151
 152 In this equation, the organic matter is stoichiometrically converted to methane, carbon
 153 dioxide and ammonia.

154 The specific methane yield expressed in litres of CH₄ per gram of Volatile Solids (VS)
 155 can thus be calculated as:

$$156 \quad B_0 = \frac{4a + b - 2c - 3d}{12a + b + 16c + 14d} * V_m \quad (2)$$

158
 159 where V_m is the normal molar volume of methane.

160 The ratio r_G of methane to carbon dioxide can therefore be computed from
 161 $n = \frac{-b + 2c + 3d}{a}$, the average carbon oxidation state in the substrate (Harris and Adams, 1979),
 162 as follows:

$$163 \quad r_G = \frac{4 - n}{4 + n} \quad (3)$$

164
 165 The biogas composition however also depends on the amount of CO₂ which is dissolved
 166 in the liquid phase through the carbonate system , and is therefore strongly related to pH
 167 [R2Q7].

168 The ammonium production yield in the digester can be evaluated using equation (1):

$$169 \quad Y_{N-NH_3} \text{ (mg gVS}^{-1}\text{)} = \frac{d * 17 * 1000}{12a + b + 16c + 14d} \quad (4)$$

170
 171 Equation (1) is a theoretical approach that allows estimation of the maximum potential
 172 yields. However, as it will be discussed later on, if the cells are directly injected into the
 173 anaerobic process, the accessibility of the intracellular components is limited for some species
 174 by the specific nature of their cell wall (Becker, 1988). On the contrary, yield can be enhanced
 175 by cell disruption after extracting a specific sub-product such as lipids for biofuel (Chisti,
 176 2007) and/or molecules of pharmaceutical interest (Spolaore et al., 2006). In this case, the
 177 remaining intracellular components become accessible for the anaerobic bacteria.

178 Table 1 (extracted from Angelidaki and Sanders (2004)) compiles specific methane yields for
 179 carbohydrates, lipids. For the specific case of proteins, the formula was calculated with the
 180 average composition in amino acids weighted by their frequency in *Chlorella vulgaris*
 181 (Becker 2007)[R2Q4]. In terms of theoretical methane potential, the higher the lipid content
 182 of the cell, the higher the potential methane yield. The high energetic content of lipids makes
 183 them attractive substrates for anaerobic digestion due to their higher gas production potential
 184 compared with carbohydrates and proteins (Cirne et al., 2007; Li et al., 2002). However, lipid
 185 hydrolysis is considered to be slower than protein and carbohydrate hydrolysis. Thus,
 186 Pavlostathis and Giraldo-Gomez (1991) calculated the minimum values of limiting generation
 187 time for anaerobic treatment of various substrates and they found values of 0.18, 0.43 and 3.2
 188 days for carbohydrates, proteins and lipids respectively. Similar results are given by Christ et
 189 al. (1999) when estimating first-order hydrolysis constants for these substrates[R2Q3].

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The raw estimate from Table 1 can be refined considering the organic composition of specific microalgae species. Using equation (1), it is possible to compute a theoretical specific methane yield associated to a theoretical ammonia release (Table 2). As expected, the species that can reach higher lipid content (*e.g. Chlorella vulgaris*) have a higher methane yield.

Obviously, in the case where lipids are extracted from algae before digestion, the potential methane yield is lower while the released ammonium is higher (See Table 4) [R1Q4].

199 2.3 Operating conditions

200 Compared to other organic substrates, studies dealing with anaerobic digestion of algae
201 are scarce. Microalgae have received less attention than macroalgae as a substrate for
202 anaerobic digestion. Two main approaches can be distinguished for unicellular algae. Either a
203 multispecific biomass is harvested from a waste water treatment pond (Chen, 1987; Chen,
204 1998; Yen and Brune, 2007), or a monospecific biomass grown in the laboratory (Asinari Di
205 San Marzano et al., 1982; Samson and LeDuy, 1982, 1986; Chen 1987; Sanchez and
206 Travieso, 1993; Munoz et al., 2005). Table 3 summarizes the experimental conditions and the
207 corresponding methane conversion yield for these reported studies. It shows that the methane
208 yield varies from 0.09 to 0.45[R1Q5] L.gVS⁻¹ depending on the species and culture
209 conditions.
210

211 2.3.1 Temperature effect

212 The increase in temperature, from 15 to 52 °C, improves the methane conversion of
213 *Spirulina maxima*, and the productivity together with the volatile solids reduction is enhanced
214 up to 35 °C (Samson and LeDuy, 1986). For a multispecific[R1Q6] algal biomass, as studied
215 by Golueke et al., (1957), a temperature increase from 35 to 50 °C can enhance the rate of
216 algae biodegradability from 5 to 10 percent. The energetic balance is then negative if we
217 consider the energy supplied for heating. However, mesophilic temperatures appear to be
218 optimal conditions as confirmed by Chen (1987) who found a maximal methane productivity
219 at 40 °C.
220

221 2.3.2 Hydraulic retention time and loading rate

222 The hydraulic and solid retention time (HRT and SRT) are key parameters in anaerobic
223 processes. They should be high enough to allow the active populations to remain in the
224 reactor, especially methanogens, and not to limit hydrolysis which is generally the limiting-
225 step of the overall conversion of complex substrates to methane. In the case of slowly
226 degradable complex organic pollutants, HRT is a deciding factor (Speece, 1996). When the
227 process is operated at low loading rate and high hydraulic retention time, the methane
228 yield[R1Q7] (L CH₄/ gVS fed) is constant and maximal. On the contrary when the maximal
229 loading rate or minimum hydraulic retention time is reached, a decrease of the yield occurs.

230 For an efficient conversion of organic matter, optimal loading rates and hydraulic
231 retention times must be chosen depending on the type or composition of the algal substrate.
232 When the cells are directly injected into the anaerobic process, accessibility of the
233 intracellular content to the anaerobic microflora is limited by the resistance of the algal cell
234 wall to hydrolysis. Thus, characteristics of the species makes the difference for a given

235 loading rate or hydraulic retention time as shown by Asinari Di San Marzano et al. (1982) and
236 Chen (1987) [R2Q6].
237

238 2.3.3 *Biogas quality*

239 The proportion of methane in the biogas produced is in a similar range (69 to 75 %) for
240 the majority of the studies, regardless of species and operating conditions. This reveals a good
241 quality of conversion of the algal organic matter into methane. The most important factor
242 impacting CH₄ proportion in the biogas is the pH, which controls the speciation of the
243 carbonate system and the release of CO₂. If the pH is high, due to high alkalinity from NH₃
244 release, then the gas content will shift more to CH₄. The oxidation state of the biomass, which
245 drives the proportion of released methane (see equation (3)), also influences the biogas
246 quality[R2Q7].

247 Since microalgae hardly contain sulphurated amino acids (Becker, 1988), their digestion
248 releases a lower amount of hydrogen sulphide than other types of organic substrates. The
249 potential presence of ammonia in the biogas, as detected by Golueke et al. (1957), due to the
250 high microalgal protein content should receive particular attention.
251

252 3 **Inhibitions induced by microalgae as substrate**

253 Two factors can have a significant impact on the methane yield and on productivity,
254 inducing an inhibition of some of the anaerobic bacterial populations. On one hand, the high
255 protein content of the algal biomass leads to a high ammonium release, thus inhibiting the
256 anaerobic microflora. On the other hand, in the case of marine species, high sodium
257 concentrations may alter the anaerobic process.
258

259 3.1 *Ammonium toxicity*

260 As described by Equation (1), and already discussed, the high nitrogen concentrations in
261 the algae leads to a significant release of ammonia during fermentation. During anaerobic
262 digestion, proteins are degraded and ammonia accumulates in the liquid phase. The pH value
263 triggers the proportion between ammonium ions (NH₄⁺) and free ammonia (NH₃). If the
264 biomass concentration in the influent is high, this will cause high NH₃ concentrations and
265 alkalinity and, as a consequence, inhibition of the process by free ammonia may occur
266 (McCarty, 1964). The unionized hydrophobic form of nitrogen diffuses passively across the
267 cell membranes where it expresses its toxicity.

268 This phenomenon has been reported in many studies (Golueke et al., 1957; Eisenberg et
269 al., 1981; Samson and LeDuy, 1982, 1983b, 1986; Chen, 1987). Anaerobic digestion of the
270 protein rich cyanobacteria *Spirulina maxima*, containing up to 60 % of proteins, releases a
271 extremely high concentration of ammonia (up to 7000 mg/L) (Samson and LeDuy, 1986).
272 Two studies, Samson and LeDuy (1982) and Sanchez and Travieso (1993), observed a strong
273 concentration of volatile fatty acids as a consequence of the toxic effect of ammonia on the
274 anaerobic flora.

275 The acetoclastic methanogen bacteria are probably among the most sensitive to NH₃
276 (Koster and Lettinga, 1984; Angelidaki and Ahring, 1993). Inhibiting concentrations vary in a
277 wide range from 1.7 to 14 g L⁻¹ and depend on several factors as the acclimation period, the
278 nature of substrate and inoculum together with operating conditions (Angelidaki and Ahring,
279 1993). Thermophilic conditions enhance the inhibition effect. It can be related to the increase
280 in free ammonia concentrations with increasing temperatures and thus with the process
281 stability (Braun et al., 1981; Angelidaki and Ahring, 1994). High concentrations of ions such

282 as Na^+ , Ca^{2+} , Mg^{2+} , which increase alkalinity and decrease the fraction of unionized NH_3 , can
283 lower the inhibition effects (Chen et al., 2008).

284 It is worth noting that methanogenic bacteria can however acclimate to high
285 concentrations of ammonium. Indeed, adaptation of the methanogenic ecosystem may
286 increase the toxicity threshold level (Chen et al, 2008), even if the methane productivity
287 remains low. For example, Koster and Lettinga (1988) reached a toxic threshold 6.2 times
288 higher after an adaptation phase [R2Q8].

289 As a consequence of high ammonium concentrations, nitrogen can be found in the biogas
290 in proportions correlated to the algal nitrogen content, as reported by Golueke et al. (1957).
291 This phenomenon has been recently highlighted for more general nitrogen rich substrates
292 (Strik et al., 2006).

293

294 3.2 *Sodium toxicity*

295 Sodium ions are required by the anaerobic microflora for its metabolism in a range from
296 0.002 to 0.004 M, but above 0.14 M, they become strongly inhibitory (Kugelman and
297 McCarty, 1965; Mc Carty, 1964; Rinzema et al., 1988). Marine microalgae require a culture
298 medium with high sodium chloride content (0.5 – 1 M). Chen (1987) observed that the
299 sodium chloride concentration has no particular effect up to 0.3 M. For 0.4 M sodium chloride
300 the methane production becomes affected and above 0.5 M toxicity is reported.

301 However, it has been proved feasible to use salt-adapted micro-organisms capable of
302 withstanding high salinities. The selection of salt-tolerant micro-organisms involves an
303 adaptation of the sludge to high salt concentrations (Chen et al., 2008). Furthermore, the
304 effluent organic loading rate and salt concentration should be equalised as far as possible, as
305 these micro-organisms are sensitive to environmental shocks, especially in anaerobiosis
306 (Lefebvre and Moletta, 2006). Hence, Aspé *et al.* (1997) adapted a marine inoculum to the
307 treatment of a fishmeal industry effluent. This explains why no inhibiting effect occurred in
308 some studies of saline waste anaerobic digestion for concentrations close to marine water
309 (Asinari Di San Marzano et al., 1982; Omil et al., 1995) [R2Q9]. In mesophilic conditions the
310 sodium turns out to be less inhibitory than in thermophilic conditions (Chen et al., 2008). The
311 presence of other ions (Ca^{2+} , K^+ , Mg^{2+}) can also play a significant, antagonistic or synergistic
312 role, on the potential toxicity of sodium (Chen et al., 2008).

313

314 4 **Anaerobic digestion enhancement**

315 The composition of the organic substrates is one of the most important factors
316 determining the methane conversion yield (Chynoweth and Isaacson, 1987). However, if the
317 algal biomass does not result from any cell disruption process, the cell walls could strongly
318 modulate this aspect by protecting the cell against the enzymes produced by the anaerobic
319 consortium, and thus reducing the cell biodegradability[R1Q8]. Indeed, some microalgae
320 species can be very resistant to hydrolysis, which drastically reduces their anaerobic
321 biodegradability (Golueke et al., 1957; Uziel, 1978; Sanchez and Travieso, 1993). During
322 their experiments (Golueke et al., 1957) identified intact cells in the digester and Uziel (1978)
323 reported the same observation even after 30 days. Sanchez and Travieso (1993) observed that
324 the chlorophyll concentration increased in the digester during the first two weeks of the
325 experiment, and was still detected after 64 days; the presence of oxygen in the biogas was
326 also reported as a consequence of the photosynthetic activity from the recalcitrant cells. For
327 example, *Scenedesmus sp* and *Chlorella sp* are known to have a recalcitrant cellulosic cell
328 wall (Okuda 2002).

329 Pre-treatment can be successfully applied in order to lower the recalcitrant organic
330 fraction and thus increase the methane conversion (Tsao, 1987).

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4.1 Pretreatment of algal biomass [R2Q10]

Pretreatment of a substrate prior to anaerobic digestion allows to significantly improve its biodegradability while acting on its physico-chemical properties: this step makes the organic matter more accessible to the anaerobic microflora and thus more easily degraded.

One generally distinguishes physical and chemical pretreatment processes. Separation techniques, concentration or dehydration, mobilize and maximize the proportion of organic matter in the fraction to be digested (Angelidaki and Ahring, 2000). Chemical treatments (acids, bases, ozonation), thermal treatment and ultrasonic lysis improve the disintegration of the most refractory organic fractions (Bonmati et al., 2001; Bougrier et al., 2006). These operations increase kinetics of production and/or methane yield.

Ultrasonic pretreatment can enhance the crude protein digestibility, as shown with experiments on the digestibility of *Chlorella vulgaris* in rats (Janczyk et al., 2007). The same effect has been observed with high pressure homogenization (Komaki et al., 1998). These techniques have turned out to be efficient means for improving the methane conversion yields (Samson and Leduy, 1983a; Chen and Oswald, 1998).

Chen and Oswald (1998) studied different pretreatments for an algal biomass produced in sewage treatment ponds. The effect of temperature, duration of the treatment, substrate concentration and sodium hydroxide addition were investigated. These pretreatments enhanced the methane specific gas production. The temperature appeared to have the most important effect, and the optimal pretreatment consisted in heating at 100 °C during 8h resulting in a 33 % improvement of methane production. Samson and Leduy (1983a) obtained the best performance for *Spirulina maxima* with a thermal pretreatment at 150 °C and a pH=11. The example of a full-scale plant for the thermal hydrolysis of sewage sludge reported by (Kepp et al., 2000), demonstrates that the improvement of the methane yield can energetically balance the thermal pretreatment.

4.2 Increasing the theoretical methane potential: a metabolic approach

The variation in the composition of microalgae is directly influenced by their growth conditions (Qiang, 2004; Spoehr and Milner, 1949). Most microalgae have the capacity, under certain conditions, to accumulate important quantities of carbon in the form of starch or lipids (Qiang, 2004). This capacity of accumulation, especially for lipids, has stimulated research aiming at the production of lipid biofuel (Chisti, 2007). The nitrogen deficiency is a well known condition to stimulate this accumulation (Ketchum and Redfield, 1949). Illman et al. (2000) evaluated the calorific value of five different species of *Chlorella* grown under low nitrogen concentrations and showed that the calorific value was directly correlated with the lipid content. The protein content was significantly reduced (Table 4) and the lipid accumulation resulted in a significant increase in the calorific value of the biomass.

A nitrogen limitation leads to an increase in the concentration of intracellular lipids (Table 4), but also to a reduction in the growth rate. From equation (2) and (4), it is possible to calculate the effect of nitrogen limitation on the cell stoichiometry, and thus on the theoretical methane potential and the theoretical ammonia release (Table 2). It is worth underlining that nitrogen limited cells have a lower protein content which leads to a lower release of ammonia. These two phenomena may therefore improve both the conversion efficiency and the stability of the process by limiting the toxic effect of ammonia.

When the anaerobic process is dedicated to digestion of cell residues after lipid extraction, biodiesel and methane are recovered, thus strongly increasing the energetic productivity of the microalgal culture (see Table 5). As mentioned by Chisti (2008), the anaerobic production of methane with cell residues is a key issue to balance both energetic and economic aspects. However, in this case the fraction of energy recovered under the form

381 of methane is reduced (theoretical methane potential is decreased) and the ammonium release
382 increased. The high ammonium concentration may then strongly limit and even jeopardize the
383 process stability. To manage this rich nitrogen substrate, a codigestion with a poor nitrogen
384 substrate is thus necessary.

385 386 4.3 Codigestion

387 The association of various substrates is a strategy to increase the performance of a
388 digester by ensuring an optimal influent composition. It has been shown to strongly enhance
389 the biogas productivity (Mata-Alvarez et al., 2000). When C/N is lower than 20 [R3Q8], there
390 is an imbalance between carbon and nitrogen requirements for the anaerobic microflora
391 (Speece 1996) leading to nitrogen release, which can become inhibiting and results in an
392 accumulation of volatile fatty acids.

393 Yen and Brune (2007) reported a significant enhancement of the methane production
394 with an addition of waste paper to algal sludge feedstock, the optimum C/N was observed to
395 be between 20 and 25. In mesophilic conditions, for a 10-days retention time and a loading
396 rate at $4 \text{ g VS L}^{-1} \cdot \text{d}^{-1}$, the blend with 50 % waste paper based on volatile solids concentration
397 doubles the methane production rate compared to direct anaerobic digestion of algal biomass
398 ($1.17 \text{ L L}^{-1} \text{ d}^{-1}$ vs. $0.57 \text{ L L}^{-1} \text{ d}^{-1}$). In the same conditions, with a loading rate of $5 \text{ gVS L}^{-1} \text{ d}^{-1}$,
399 the algal sludge mixed with 60 % of waste paper, lead to a maximum methane production rate
400 of $1.61 \text{ L L}^{-1} \text{ d}^{-1}$ [R1Q10].

401 The improved performance with such an approach are confirmed by Chen (1987) who
402 associated algae with effluent from canning facility and protein-extracted algae. In this case
403 the optimal methane specific gas production was reached for a C/N ratio between 25 and 35.
404 In these studies, the optimal C/N ratio was found between 20 and 35. This value is close to the
405 described range known to have a positive effect on the methane yield (Angelidaki et al.,
406 2003). Lower ratios lead to potential inhibition due to the presence of free ammonia whereas
407 higher ratios may lead to potential nitrogen limitations. By increasing the C/N ratio (from 4.2
408 to 6.2) using sewage sludge, Samson and LeDuy (1983b) enhanced both methane yield and
409 productivity during the codigestion of *Spirulina maxima*. Some co-substrate can have a co-
410 effect in the sense that they stimulate enzymatic synthesis that can also improve the anaerobic
411 digestion yield [R3Q9]. Indeed, Yen and Brune (2007) showed an increase in the cellulase
412 activity stimulated by the specific nature of the waste paper. It probably had a positive effect
413 on the digestion of algal cell walls and therefore on the anaerobic digestion itself. Finally,
414 codigestion leads to the dilution of certain toxic compounds maintaining them under their
415 toxic threshold.

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418 5 Biogas purification[R2Q13] [R3Q4]

419 Autotrophic photosynthesis of microalgae supposes a continuous CO_2 consumption
420 during the light phase. This property advantageously points to these fast-growing organisms
421 as a promising technology for CO_2 fixation [R2Q13][R3Q4] (Li, 2008; Wang, 2008)[R3Q5].
422 In the 60s, Oswald and Golueke (1960) demonstrated the feasibility of mitigating gas
423 effluents resulting from a power plant with a high-rate pond. Furthermore, the addition of CO_2
424 in algal ponds enhances algal growth (Olaizola, 2003; Doucha et al., 2005) provided that pH
425 is regulated. It also maintains a low pH that decreases the gaseous ammonia emission
426 (Heubeck et al., 2007). The CO_2 concentration in a biogas in the range 30 to 50 % appears to
427 be compatible with the toxicity and inhibition thresholds reported for the commonly exploited
428 species (Maeda et al., 1995).

429 The filtration of the biogas by algal cultures supplies CO₂ to the culture, thus enhancing
430 the algal growth and productivity (Travieso et al., 1993). Moreover, methane does not induce
431 any toxicity on the growth of algae (Travieso et al., 1993; Mandeno et al., 2005; Heubeck et
432 al., 2007).

433 Biogas purification through the algal culture can be very efficient. Indeed, Travieso et al.
434 (1993) evaluated the capacity of a culture of *Arthrospira sp.* [R1Q11] to filter a biogas
435 resulting from the digestion of molasses of a sugar refinery. The influent biogas was
436 composed of 55 to 71 % of CH₄[R2Q14]. Once the microalgal culture had filtered the biogas,
437 the methane concentration had increased up to 88-97 % CH₄ while CO₂ had decreased down
438 to 2.5 - 11.5 % CO₂. In another study, (Mandeno et al., 2005) a counter-current pit was used
439 for the treatment of a synthetic biogas composed with 60 % N₂ and 40 % CO₂. A 90 %
440 decrease in CO₂ was observed, while the produced oxygen did not exceed 6 %. Finally the
441 nitrogen gas content increased up to 95 %.

442 Biogas produced from anaerobic digestion of organic matter is mainly composed of
443 methane and carbon dioxide, but also in a smaller fraction, of hydrogen sulphide, dinitrogen,
444 dihydrogen and other volatile compounds (Rasi et al., 2007). As already discussed, with a
445 high nitrogen content, the biogas can also contain ammonia (Strik et al., 2006).

446 The effects of the minor components in the biogas on the algae vary from one study to
447 another. They seem highly related to the algal species and concentrations, their state and the
448 presence of other inhibiting compounds. Some species have a higher tolerance towards NO_x
449 and SO_x components (Michiki, 1995; Chae et al., 2006). Olaizola (2003) showed that the
450 main effect of SO_x and NO_x is indirect due to their acidity since they lead to a drop in pH. A
451 pH control procedure therefore strongly limits their effect on the algae.

452 Travieso et al. (1993) showed that the concentration of H₂S in the gas decreased after
453 filtration by *Arthrospira sp.* from 1% to 0.3 - 0.4 %. This reduction was however mainly
454 attributed to the gas solubility. The quality of biogas is a key issue for the longevity and
455 efficiency of the thermic process converting methane into energy, and the algae can
456 significantly reduce the cost associated to biogas filtration. Moreover algae increase the
457 methane content which facilitates the biogas energetic conversion (Heubeck et al., 2007).
458 However, because of certain corrosive or toxic compounds which remain in the biogas even
459 after algal filtration and cannot be removed, the biogas still requires a necessary stage of
460 purification.

461

462 **6 Is it worth to recover lipids from an energetic point of view?**

463

464 In this section we provide several elements in order to compare the direct strategy
465 (methanizing the whole biomass) and the indirect one, including lipid recovery and methane
466 production with the algal waste. Table 5 highlights the comparison between these two scenarii.
467 For an algal lipid content lower than 40%, the energetic added value when recovering lipids is
468 lower than 21% of the recovered energy. However, the energetic cost of biomass harvesting
469 and lipid recovery is probably higher than 30% of the recovered energy, especially since most
470 of the existing techniques involve biomass drying (Carlsson et al., 2007), while the direct
471 strategy would involve only a sedimentation and preconcentration stage in a settler. This put
472 an emphasis on the idea that direct energy recovery can be of interest in the case where the
473 lipid content is lower than 40%. This point is consolidated when considering the
474 triacylglycerols which are the actual substrate to produce biodiesel. They may represent only
475 a small fraction of total lipids when no nitrogen limitation is induced, and thus in the
476 situations when lipid content is low (Rodolfi et al. 2008). This assumption is further
477 confirmed when the productivity is taken into account. A nitrogen limitation induces a strong

478 decrease in growth rate (Droop, 1983). Consequently the increase in the lipid content is
479 generally not compensated and eventually productivity is decreased (Rodolfi et al. 2008).
480 Hence, even the energetic advantage for the indirect scenario appearing in Table 5 for algal
481 lipid contents higher than 40% could be strongly reduced due to a decrease in
482 productivity.[R2Q11] [R3Q1] [R2Q3]

483
484

485 7 Conclusion

486

487 The perspective of large scale microalgae production for CO₂ fixation and/or lipid fuel
488 production assumes large amounts of fertilizers, especially if compared to terrestrial plants.
489 Nitrogen and phosphorus remineralisation using anaerobic digestion can support this strong
490 nutrient requirement and moreover recover additional energy through methane. However this
491 operation is not straightforward and three main aspects have to be considered:

492 1- In the case of marine algae, sodium can inhibit anaerobic digestion. However this issue
493 has already been addressed and adapted bacteria seem to be efficient.

494 2- The release of nitrogen is toxic at high concentrations. This effect should be
495 exacerbated if pH increases. To control and limit the risk of destabilizing the anaerobic
496 process by free ammonia, several strategies have been investigated. A microalgae codigestion
497 with a nitrogen poor substrate can be an answer to this problem. The second answer would be
498 to use species with a higher C/N ratio, and to apply culture conditions that maximise this
499 ratio. However the strategy needs to be adapted, depending whether lipids have been
500 recovered in a preliminary step.

501 3- If the cell lipid content does not exceed 40 %, the anaerobic process appears to be the
502 optimal strategy on an energy balance basis, for the energetic recovery of cell biomass.

503 This study justifies the exploration of the potential of the direct scenario, without lipid
504 recovery, and provides new motivations to more accurately identify the lipid content
505 threshold under which recovering the oil is no more relevant from an energetic point of view.

506 Explored about fifty years ago, the promising integration process coupling anaerobic
507 digestion and microalgal culture deserves sustained research and development efforts and will
508 probably re-emerge in the coming years either as a mandatory step to support large scale
509 microalgal cultures or as a stand alone bioenergy producing process.[R2Q15] [R3Q3]

510

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711 **Table 1**
 712 Specific methane yield for three types of organic compounds

Substrate	Composition	L CH ₄ g VS ⁻¹
Proteins	C ₆ H _{13.1} O ₁ N _{0.6}	0.851
Lipids	C ₅₇ H ₁₀₄ O ₆	1.014
Carbohydrates	(C ₆ H ₁₀ O ₅) _n	0.415

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721 **Table 2**
 722 Gross composition of several microalgae species (Becker, 2004) and calculated (using
 723 equation (1)) theoretical methane potential and theoretical ammonia release during the
 724 anaerobic digestion of the total biomass

Species	Proteins (%)	Lipids (%)	Carbohydrates (%)	CH ₄ [R1Q12] (L CH ₄ g VS ⁻¹)	N-NH ₃ (mg gVS ⁻¹)
<i>Euglena gracilis</i>	39-61	14-20[R2Q5]	14-18	0.53-0.8	54.3-84.9
<i>Chlamydomonas reinhardtii</i>	48	21	17	0.69	44.7
<i>Chlorella pyrenoidosa</i>	57	2	26	0.8	53.1
<i>Chlorella vulgaris</i>	51-58	14-22	12-17	0.63-0.79	47.5-54.0-
<i>Dunaliella salina</i>	57	6	32	0.68	53.1
<i>Spirulina maxima</i>	60-71	6-7	13-16	0.63-0.74	55.9-66.1
<i>Spirulina platensis</i>	46-63	4-9	8-14	0.47-0.69	42.8-58.7
<i>Scenedesmus obliquus</i>	50-56	12-14	10-17	0.59-0.69	46.6-42.2

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727 **Table 3**
 728 Experiments with anaerobic digestion of microalgae species and algal sludge: substrate
 729 characteristics, methane yield [R1Q14] and process conditions

Reactor	Substrate	T ^a (°C)	HRT ^d (d)	Loading rate (gVS. L ⁻¹ .j ⁻¹)	Methane yield [R1Q14] (L CH ₄ g VS ⁻¹)	CH ₄ (% vol)	References
Batch 11 L	Algae sludge (<i>Chlorella</i> – <i>Scenedesmus</i>)	35-50	3-30	1.44 – 2.89	0.17 – 0.32	62 - 64	(Golueke et al., 1957)
	Algal biomass	35	28	1	0.42	72	
	<i>Spirulina</i>	35	28	0.91	0.32 – 0.31		(Chen, 1987)
	<i>Dunaliella</i>	35	28	0.91	0.44-0.45		
	<i>Tretraselmis</i> (fresh)	35	14	2	0.31	72-74	
CSTR ^b 2-5 L	<i>Tretraselmis</i> (dry)	35	14	2	0.26	72-74	(Asinari Di San Marzano et al., 1982)
	<i>Tretraselmis</i> (dry) + NaCl 35g/L	35	14	2	0.25	72-74	
Batch 5 L	<i>Chlorella vulgaris</i>	28-31	64	-	0.31-0.35 ^d	68-75	(Sanchez and Travieso, 1993)
Semi continuous (daily fed) 10 L	<i>Spirulina maxima</i>	35	33	0.97	0.26	68-72	(Samson and LeDuy, 1982)
Fed Batch 2 L	<i>Spirulina maxima</i>	15-52	5-40	20-100	0.25-0.34	46-76	(Samson and LeDuy, 1986)
CSTR ^b 4L	<i>Chlorella-Scenesmus</i>	35	10	2-6	0.09-0.136	69	(Yen and Brune, 2007)

^a Temperature

^b Continuous Stirred-Tank Reactor

^c estimated from data given in L CH₄.gCOD-1 using a COD/VS ratio of 1.5 (where COD is the Chemical Oxygen Demand)

^d Hydraulic Retention Time

[R1Q13]

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737 **Table 4**
 738 Effect of low nitrogen growth conditions on the composition of five *Chlorella* species and
 739 estimation of the theoretical methane potential and theoretical ammonia release (in brackets:
 740 computed theoretical methane potential and ammonia release of the residual biomass after
 741 lipid extraction).
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Species	Growth conditions	Proteins (%)	Lipids (%)	Carbohydrates (%)	Calorific value (Kj/g)	CH ₄ (l CH ₄ g VS ⁻¹)	N-NH ₃ (mg g VS ⁻¹)
<i>C.vulgaris</i>	-	29	18	51	18	0.64 (0.56)	27.0 (32.9)
<i>C.vulgaris</i>	Low N	7	40	55	23	0.69 (0.48)	6.5 (10.9)
<i>C.emersonii</i>	-	32	29	41	21	0.74 (0.62)	29.8 (42.0)
<i>C.emersonii</i>	Low N	28	63	11	29	0.92 (0.76)	26.1 (70.5)
<i>C.protothecoides</i>	-	38	11	52	19	0.65 (0.60)	34.5 (39.8)
<i>C.protothecoides</i>	Low N	36	23	41	24	0.71 (0.62)	33.5 (43.6)

[R1Q16]

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746 **Table 5 [R3Q1] [R3Q3]**

747 Energetic content of microalgae according to two scenarii. S1: anaerobic digestion of the
 748 whole biomass, S2: digestion of biomass residues after lipids extraction.
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Species	Growth conditions	S1: Anaerobic digestion of the whole algal biomass	S2: Anaerobic digestion of algal biomass residues			Energetic added value with lipid recovery
		Methane ^a (kJ/g VS)	Methane ^a (kJ/g VS)	Lipids ^b (kJ/g VS)	Total energy (kJ/g VS) ^a	Additional energy (kJ/g VS)
<i>C.vulgaris</i>	-	23.0	20.1	6.6	26.7	3.7
<i>C.vulgaris</i>	Low N	24.9	17.2	14.7	32.0	7.1
<i>C.emersonii</i>	-	26.4	22.4	10.7	33.1	6.6
<i>C.emersonii</i>	Low N	33.1	27.6	23.2	50.8	17.7
<i>C.protothecoides</i>	-	23.4	21.8	4.1	25.8	2.4
<i>C.protothecoides</i>	Low N	25.5	22.2	8.5	30.7	5.2

750 ^a: computed with a methane calorific value of 35.6 kJ/L.

751 ^b: computed with the calorific value of rapeseed crude oil : 36.87 MJ/t ,(Labeckas, 2009).

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