



**HAL**  
open science

## **Integrating microalgae production with anaerobic digestion: a biorefinery approach**

Enrica Uggetti, Bruno Sialve, Eric Trably, Jean-Philippe Steyer

### ► **To cite this version:**

Enrica Uggetti, Bruno Sialve, Eric Trably, Jean-Philippe Steyer. Integrating microalgae production with anaerobic digestion: a biorefinery approach. *Biofuels, Bioproducts & Biorefining*, 2014, 8 (4), pp.516-529. 10.1002/bbb.1469 . hal-02639931

**HAL Id: hal-02639931**

**<https://hal.inrae.fr/hal-02639931>**

Submitted on 9 Aug 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **INTEGRATING MICROALGAE PRODUCTION WITH ANAEROBIC DIGESTION:**  
2 **A BIOREFINERY APPROACH**

3

4 Enrica Uggetti\*, Bruno Sialve, Eric Trably, Jean-Philippe Steyer

5

6 INRA, UR0050,

7 Laboratoire de Biotechnologie de l'Environnement,

8 Avenue des Etangs, Narbonne, F-11100, France.

9

10

11 \*Corresponding author: Enrica Uggetti

12 Tel: +33 468 42 51 51

13 Fax: +33 468 42 51 60

14 E-mail address: [enrica.uggetti@supagro.inra.fr](mailto:enrica.uggetti@supagro.inra.fr)

15 **Abstract**

16 In the energy and chemical sectors, alternative production chains should be considered  
17 in order to simultaneously reduce the dependence on oil and mitigate climate change.

18 Biomass is probably the only viable alternative to fossil resources for production of  
19 liquid transportation fuels and chemicals since, besides fossils, it is one of the only  
20 available sources of carbon rich material on earth. Over recent years, interest towards  
21 microalgae biomass has grown in both fundamental and applied research fields. The

1 biorefinery concept includes different technologies able to convert biomass into added  
2 value chemicals, products (food and feed) and biofuels (biodiesel, bioethanol,  
3 biohydrogen). As in oil refinery, a biorefinery aims at producing multiple products,  
4 maximizing the value derived from differences in biomass components, including  
5 microalgae. This paper provides an overview of the various microalgae-derived  
6 products, focusing on anaerobic digestion for conversion of microalgal biomass into  
7 methane. Special attention is paid to the range of possible inputs for anaerobic digestion  
8 (microalgal biomass and microalgal residue after lipid extraction) and the outputs  
9 resulting from the process (e.g. biogas and digestate). The strong interest for microalgae  
10 anaerobic digestion lies in its ability to mineralize microalgae containing organic  
11 nitrogen and phosphorus, resulting in a flux of ammonium and phosphate that can then  
12 be used as substrate for growing microalgae or that can be further processed to produce  
13 fertilizers. At present, anaerobic digestion outputs can provide nutrients, CO<sub>2</sub> and water  
14 to cultivate microalgae, which in turn, are used as substrate for methane and fertilizer  
15 generation.

16

17 **Keywords:** Microalgae, Biorefinery, Co-products, Anaerobic Digestion, Methane  
18 production.

## 19 **1. Introduction**

20 Nowadays, the important increase of the oil demand is placing an enormous pressure on the  
21 finite supply of fossil fuel-derived energy and chemicals. For this reason, the development of  
22 alternative production chains in the energy and chemical sectors is necessary in order to  
23 simultaneously reduce the dependence on oil and mitigate climate change.

1 Plant-based raw materials (i.e. biomass) have the potential to replace a large fraction of fossil  
2 resources as feedstock for industrial production. Due to its high carbon content, biomass is a  
3 suitable alternative to fossil resources for production of liquid transportation fuels and  
4 chemicals. In addition, biomass resources are locally available in many countries and their use  
5 could largely contribute to reduce national dependence on imported fossil fuels.<sup>1</sup>  
6 Beyond their energetic value, microalgae have been widely investigated as sources of  
7 chemicals, cosmetics and health products, animal and human feed. In fact, photosynthetic  
8 organisms such as higher plants, algae, and cyanobacteria are capable of using sunlight and  
9 carbon dioxide to produce valuable organic molecules, such as carbohydrates, lipids,  
10 pigments, fibers, etc. Over the recent years, the interest for microalgae biomass has increased  
11 in both fundamental and applied research fields aiming at producing biofuels and  
12 biochemicals. This paper provides an overview of the various products obtained from  
13 microalgae biomass, with a special focus on anaerobic digestion for methane and fertilizer  
14 production.

## 15 **2. Microalgae biorefinery**

16 The biorefinery concept consists in different technologies able to convert any type of biomass  
17 to value-added products, biofuels and chemicals. This concept is derived from the petroleum  
18 refinery, which uses petroleum to produce multiple fuels and products with applications in  
19 various industries. As in oil refinery, a biorefinery aims at generating multiple end-products,  
20 and maximizing the value derived from differences in biomass components. In order to design  
21 an efficient and cost effective biorefinery, an important stage is the provision of a renewable,  
22 consistent and regular supply of feedstock (raw materials used in biorefinery). In this context,  
23 microalgae, including all unicellular and simple multi-cellular microorganisms, such as  
24 prokaryotic microalgae (e.g. cyanobacteria *Chloroxybacteria*), eukaryotic microalgae (e.g.

1 green algae (*Chlorophyta*), red algae (*Rhodophyta*) and diatoms (*Bacillariophyta*) play an  
2 important role as biorefinery feedstock.<sup>2</sup> These photosynthetic organisms can be cultivated in  
3 freshwater, seawater and wastewater, and they can be farmed on non-arable land. Moreover,  
4 certain microalgae can tolerate and adapt to a wide variety of environmental conditions (in  
5 terms of pH, temperature, light, etc.) and can be produced all year round. Table 1 compares  
6 the biomass productivity of microalgae (up to 70 ton dry weight (DW) per ha per year) and  
7 conventional agricultural crops together with their raw energy productivity.

8 Microalgae are typically composed by proteins, carbohydrates, lipids, and other valuable  
9 components (e.g. pigments, anti-oxidants, fatty acids and vitamins) (Table 2). These  
10 components are valuable for a wide range of applications. The carbohydrates present in  
11 microalgae are considered as an appropriate feedstock for microbial growth and generation of  
12 various fermentation products. The high lipid content in algal biomass makes it promising for  
13 biodiesel production. However, special attention to the fractions of lipids stored in microalgae  
14 should be paid, and unsaturated fatty acids from microalgae may need to be hydrogenated to  
15 improve fuel properties. Finally, the related long-chain fatty acids, pigments and proteins  
16 have their own nutraceutical and pharmaceutical applications. However, the technology for  
17 the commercial production of microalgae bioproducts is still being under development and  
18 investigation. More particularly, additional efforts should be made to reduce the operating  
19 costs, that are essentially associated with algal biomass growth (e.g. nutrients, light and CO<sub>2</sub>  
20 distribution), harvesting (i.e. isolation of the biomass from the culture, dilution or  
21 concentration of algae to suitable levels for further processing), and downstream processing  
22 obtaining valuable products or subproducts.

23 In this sense, even though economics are strictly correlated with the biochemical composition  
24 of the biomass, Williams and Laurens (2010)<sup>5</sup> emphasized that the “biofuel only” option is  
25 unlikely to be economically viable and other sources of revenue are needed to make the

1 system profitable. For this reason, the main challenge prior to any biorefinery development is  
2 the optimization of efficient and cost effective production of transportation biofuels,  
3 biomaterials and biochemicals, by using all biomass components as co-products.

#### 4 ***2.1 Pharmaceuticals, food and feed***

5 Many microalgae naturally contain omega-3 fatty acids which can be purified to provide a  
6 high-value food supplement.<sup>6</sup> In addition, eicosapentanoic acid (EPA) as well as  
7 decosahexaenoic acid (DHA) have pharmaceutical applications in the treatment of heart and  
8 inflammatory diseases (e.g. asthma, arthritis, headache and psoriasis) as well as in the  
9 prevention and cure of cancer, AIDS, to control and lower cholesterol, or to boost the immune  
10 system and body detoxification.<sup>7</sup>

11 The antioxidants produced from microalgae to protect the photosynthetic cells from oxidative  
12 stress can be used in the medical field to limit or prevent health problems, such as  
13 atherogenesis, cancer, neurodegenerative diseases, infant retinopathy, muscular degeneration  
14 and renal failure.<sup>8</sup> In addition, hydrocarbons contained in microalgae can replace the  
15 paraffinic and natural waxes in the production of facial masks for the cosmetic industry.

16 Microalgae are also used in pharmaceuticals or in cosmetics as a source of chlorophyll  
17 pigment and they are currently gaining importance as a food additive due to their strong  
18 naturally green color. Traditionally the above mentioned compounds have been obtained by  
19 solvent extraction. However many researchers are nowadays focusing on more sustainable  
20 extraction techniques. As an illustration, supercritical CO<sub>2</sub> extraction was recently applied for  
21 successful lipid extraction on *Botryococcus braunii*, *Chlorella vulgaris*, *Dunaliella salina*  
22 and the cyanobacteria *Arthrospira (Spirulina) maxima*.<sup>9</sup> However, CO<sub>2</sub> can only extract the  
23 neutral lipid fraction and, in order to achieve higher yields, alternative extraction techniques  
24 combined with polar extraction solvents (e.g. microwave-assisted extraction, ultrasound-  
25 assisted extraction, extraction with pulsed electric field, bead-beating-assisted extraction,

1 Soxhlet extraction, pressurized fluid extraction, and others) were also reported in the  
2 literature, each having their own advantages and disadvantages.<sup>10</sup>

3 Many algal species have been also examined by various researchers for their biochemical  
4 compositions to be suitable as substitute or primary livestock feed. Indeed, it has been  
5 reported that microalgae can play a key role in high-grade animal nutrition food, from  
6 aquaculture to farm animals. Comprehensive nutritional and toxicological evaluations  
7 demonstrated the suitability of algae biomass as a valuable feed supplement or substitute in  
8 conventional animal feed sources.<sup>11</sup>

## 9 **2.2 Fuel products**

### 10 **2.2.1 Biodiesel**

11 The viability of microalgae for biodiesel production has been investigated by a number of  
12 studies.<sup>12, 13, 14</sup> Authors pointed out that, in spite of a certain dependence of the oil yield of the  
13 algal strain, the oil content of microalgae is generally much higher than for other plant crops.  
14 In fact, many species of algae produce amounts of lipids as high as 50–60% of their dry  
15 weight. Various methods for lipid extraction from microalgae were reported in literature, the  
16 most common methods being expeller/oil press, liquid–liquid extraction (solvent extraction),  
17 supercritical fluid extraction and ultrasound techniques.<sup>13</sup>

18 Concerning the species the most suitable for biodiesel production, *Botryococcus braunii*,  
19 *Chlorella vulgaris*, *Nannochloropsis* sp., *Nitzschia laevis*, *Parietochloris incise* and  
20 *Schizochytrium* sp. have oil contents higher than 50% dry weight.<sup>15</sup> However, only few strains  
21 are nowadays commercially produced and there is a strong need for screening for new strains  
22 or modifying the existing strains in order to reach an optimal lipid content for efficient  
23 biodiesel production.<sup>16</sup>

### 1 2.2.2 Bioethanol

2 Bioethanol from algae represents a significant potential due to their low percentage of lignin  
3 and hemicellulose compared to other lignocellulosic plants and to the important amount of  
4 carbohydrates, typically galactose (23%) and glucose (20%) which are energy-rich  
5 compounds<sup>17</sup> In fact, certain species of microalgae have the ability of producing high levels of  
6 carbohydrates instead of lipids as reserve polymers. The starch accumulated within the  
7 chloroplasts or the cytoplasm<sup>18</sup> is a source of carbohydrates that can be extracted to produce  
8 fermentable sugars. Bioethanol from biomass could therefore be obtained by means of  
9 biochemical processes (i.e. fermentation), thermo-chemical processes or gasification. The  
10 microalgae *Chlorella vulgaris*, more particularly, has been considered as a promising  
11 feedstock for bioethanol production as it can accumulate up to 37% (dry weight) of starch.<sup>19</sup>  
12 *Chlorococum* sp. was also used as a substrate for bioethanol production under different  
13 fermentation conditions.<sup>19</sup> Bioethanol can be produced directly from the microalgae biomass  
14 or from the exhausted biomass following lipid extraction. For example, Harun et al. (2009)<sup>20</sup>  
15 tested the effect of different fermentation conditions and parameters on accumulation of  
16 bioethanol and found that the lipid-extracted microalgae gave 60% higher ethanol  
17 concentrations than the dried and intact microalgae. In this way, microalgae could be used for  
18 the production of both lipid-based biofuels and for ethanol biofuels from the same biomass,  
19 thus increasing their overall economic value.

20 In addition, CO<sub>2</sub> produced as by-product from the fermentation process can be recycled as  
21 carbon source for further microalgae cultivation. This aspect is discussed in further details  
22 below.

### 23 2.2.3 Biohydrogen

24 In the case of biohydrogen production, microalgae can either produce themselves  
25 biohydrogen after derivation of their photosynthetic metabolism, or be used as feedstock for



1 further biohydrogen production by microbial dark fermentation.<sup>21,22</sup> For one side, certain  
2 photosynthetic microalgae and cyanobacteria are capable of directly producing biohydrogen  
3 through photobiolysis involving the oxidation of ferredoxin by the hydrogenase enzyme, but  
4 only when the cellular metabolism is restricted, ie. under medium (S) starvation and low light  
5 intensity. In that case, the reduced ferredoxin are reoxidized by transferring their electrons to  
6 the hydrogenase. However, hydrogenases directly compete with many other metabolic  
7 processes for the partitioning of electrons, and are strongly inhibited by the presence of the  
8 oxygen, produced concomitantly by photosynthesis. To avoid such inhibition, a two steps  
9 growth, so-called indirect biopholysis, is recommended where the microalgae grows in the  
10 first stage with no light or medium limitation followed by hydrogen production under medium  
11 (S) starvation and lower light intensity.

12 In this context, a significant amount of recent research on microalgae photobiohydrogen  
13 production has focused on the optimization of process operation as well as the identification  
14 of more robust hydrogenase activities, and especially on oxygen-tolerant hydrogenases.<sup>23, 24</sup>

15 In addition, certain purple non sulfur (PNS) bacteria, e.g. *Rhodobacter* sp. or *Rhodospirillum*  
16 sp., can also produce biohydrogen by photofermentation.<sup>22</sup> This consists in the fermentation  
17 of organic compounds (sugars, volatile fatty acids, alcohols) under illumination but in absence  
18 of nitrogen in the growth medium. In these microorganisms, the organic compounds are  
19 oxidized by a fermentative pathway, ie. under anoxygenic conditions, and the protons are  
20 reduced by a nitrogenase, when the cells are under nitrogen starvation.<sup>25</sup> In fact, nitrogenase  
21 has a high affinity to nitrogen and any nitrogen source in the medium can cause severe  
22 inhibition of the phtotofermentative production of biohydrogen. Moreover, this cellular  
23 mechanism requires high amount of energy in the form of ATP molecules, and therefore with  
24 low hydrogen yields (<1.5 moleH<sub>2</sub> per mole glucose).<sup>25</sup>

1 On the other side, microalgae can also be used as substrate for dark fermentation to produce  
2 hydrogen. The hydrogen productivities are considerably higher with microbial dark  
3 fermentation when considering the use of algae as substrate than through photobiological  
4 pathways. For this reason, dark fermentative H<sub>2</sub> production from microalgal biomass has  
5 received increasing attention over the past few years. It was shown that the use of microalgae  
6 *Chlamydomonas* spp., *Chlorella* sp., *Dunaliella tertiolecta* and *Scenedesmus* spp. as feedstock  
7 led to hydrogen yields ranging between 17 and 114 mLH<sub>2</sub>/gVS (volatile solids).<sup>26</sup>  
8 These results are consistent or even competitive with the biohydrogen yields obtained  
9 from terrestrial plants and agricultural wastes, as previously reported by Guo et al. (2010)<sup>27</sup> As  
10 pointed out by Cheng et al. (2011), the algal biomass is very suitable as feedstock for  
11 biohydrogen by dark fermentation since several strains of microalgae could accumulate  
12 carbohydrates in significant amounts.<sup>28</sup> Yang et al. (2010) suggested also to use the residual  
13 microalgal biomass after oil extraction processes to produce hydrogen, which suits perfectly  
14 with a concept of environmental biorefinery.<sup>29</sup>

15

#### 16 2.2.4 Biogas

17 Anaerobic digestion is a common process to treat organic waste in most of the developed  
18 countries across the world. During the past few years, it has been largely implemented  
19 because of the increase in the economic subsidies for generation of electricity from biogas. In  
20 certain countries (such as Germany and Sweden), biogas is also used as transportation biofuel,  
21 after purification upgrading to biomethane. In the following, we will focus on the anaerobic  
22 conversion of microalgae biomass to methane. Special attention will be paid to the vast range  
23 of possible inputs on anaerobic digestion and outputs resulting from the process (e.g. biogas  
24 and digestate).

### 1 **3. Anaerobic digestion of microalgae**

2 Anaerobic digestion is a microbial process of degradation and stabilization of organic  
3 materials under anaerobic conditions, leading to the formation of biogas and digestate (with  
4 liquid and solid phases). The process is carried out by heterogeneous microbial populations  
5 involving multiple biological and substrate interactions. Anaerobic digestion (also called  
6 methanogenic fermentation, or methanogenesis) is widely applied to the treatment of liquid  
7 wastewaters (in particular for the treatment of effluents from food, pulp, paper and chemical  
8 industries) and solid waste originating from agriculture (e.g. manure and plant residues) or  
9 from urban activities such as sewage sludge in wastewater treatment plants and the organic  
10 fraction of municipal solid wastes (OFMSW)).

#### 11 ***3.1 Substrate for anaerobic digestion***

##### 12 *3.1.1 Microalgae*

13 During the past years, interest has grown in favor of anaerobic digestion of microalgal  
14 biomass, leading to studies on various freshwater and marine microalgae, and using different  
15 process combinations. Over the past five years, investigations tested a wide range of process  
16 temperatures, reactor configurations, pretreatment methods as well as the use of co-substrates.  
17 Due to the specific cell wall properties, anaerobic digestion efficiency is often strain  
18 specific.<sup>30,31</sup> Indeed, a significant variability of the methane yield (from 140 up to 400  
19 mLCH<sub>4</sub>/gVS<sub>influent</sub>) is observed in the literature, likely due to different operating conditions of  
20 the digester (i.e. bioreactor type, hydraulic retention time and the digestion temperature<sup>30</sup>) in  
21 combination with microalgal strain selection and cultivation conditions that are responsible  
22 of variations in protein, carbohydrate and lipid cellular contents, as well as cell wall  
23 structure.<sup>32</sup>

24 Recently, Frigon et al. (2013)<sup>33</sup> tested under similar operating conditions a selection of 15  
25 freshwater and 5 marine microalgae in order to identify a microalgal strain suitable for large

1 scale production of methane. The Biochemical Methane Potential (BMP) tests were  
2 performed using a microalgae:sludge inoculum ratio of 2:1 based on volatile solids  
3 concentration. Results showed no significant difference in the maximum methane yield  
4 between freshwater microalgae (330 mLCH<sub>4</sub>/gVS<sub>influent</sub>) and marine microalgae (300  
5 mLCH<sub>4</sub>/gVS<sub>influent</sub>) although it varied greatly within the tested strains (230-410  
6 mLCH<sub>4</sub>/gVS<sub>influent</sub>).

7 Moreover, the anaerobic digestion process can be inhibited by ammonia issued from  
8 biological degradation of nitrogenous matter and by sulfide causing toxicity effects on various  
9 bacterial groups.<sup>32, 34</sup> Toxic effects on AD can also be induced by high sodium levels when  
10 marine microalgae are used as a substrate. Optimum sodium concentrations are around 230-  
11 350 mg Na<sup>+</sup>/L, while inhibitory effects were reported at concentrations higher than 3,500 mg  
12 Na<sup>+</sup>/L.<sup>34</sup>

13 The wide and recent interest of the scientific community on microalgae anaerobic digestion is  
14 related to its ability to mineralize algal waste containing high amount of organic nitrogen and  
15 phosphorus, resulting in a flux of ammonium and phosphate that can then be reused as  
16 substrate for microalgae cultivation<sup>35,36</sup> or further processed to obtain fertilizers. Similarly to  
17 light, CO<sub>2</sub> and water, the lack of nutrients can be an important obstacle preventing the scaling  
18 up of microalgae biorefinery technologies.<sup>5</sup> Here, these nutrients are partially supplied by the  
19 outlet of the anaerobic digester. In this context, the microalgae grown in wastewaters, together  
20 with other residues, can be used as a digestion substrate and the digestion outputs (nutrients,  
21 water and CO<sub>2</sub>) can provide substrates for microalgal culture (Figure 1). Then, the methane  
22 produced from the anaerobic digestion process can be converted to generate transportation  
23 biofuel, heat, or electricity used in microalgae processing.

24

25

### 1 3.1.2 Co-digestion

2 The carbon/nitrogen (C/N) ratio is an important factor for guarantying the stability of the  
3 anaerobic digestion process. A C/N ratio of 25 to 32 was reported to have a positive effect on  
4 the methane yield.<sup>37</sup> At lower C/N ratios, the risk of excess in nitrogen, not needed for  
5 biomass synthesis, becomes inhibitory. On the contrary, a very high C/N ratio would lead to  
6 nitrogen deficiency for biomass synthesis. Hence, co-digestion can be an alternative to  
7 improve process performance by adding a secondary substrate that supplies nutrients lacking  
8 in the initial substrate. Combination of two or more substrates could create a synergistic effect  
9 by alleviating the nutrient imbalance and, in turn, attenuating the inhibition effects of the  
10 individual substrate. As previously mentioned, microalgal biomass generally contains high  
11 amounts of nitrogen, therefore a carbon-rich co-substrate could be added to facilitate the  
12 methane conversion process. For example, the addition of carbon-rich paper waste to a  
13 mixture of *Scenedesmus* spp. and *Chlorella* spp. resulted in an improved methane yield and  
14 increased cellulase activity.<sup>38</sup> Similarly, Gonzalez et al. (2011)<sup>39</sup> detected a significant  
15 increment of the methane yield when microalgae biomass was digested with swine manure as  
16 co-substrate.

### 17 3.1.3 Microalgae residue

18 The microalgae lipid extraction process results in a biomass residue which accounts for  
19 approximately 65% of the harvested biomass.<sup>40</sup> This can be considered as a waste with a  
20 certain disposal cost that will further increase the already unfavorable economics for biodiesel  
21 production from microalgae.<sup>41</sup> However, algal residues contain significant quantities of  
22 proteins and carbohydrates, which could undergo anaerobic digestion to produce biogas.<sup>42</sup>  
23 Yang et al. (2011)<sup>43</sup> reported a methane yield of 390 mLCH<sub>4</sub>/gVS<sub>influent</sub> from residual  
24 *Scenedesmus* biomass derived from oil extraction processes.

1 However microalgae biomass residues generated after lipid extraction may cause more severe  
2 ammonia inhibition than the whole algae, due to their higher protein contents.<sup>42</sup> As already  
3 pointed out, this can be moderated through co-digestion to increase the carbon:nitrogen ratio.  
4 An illustration, co-digestion of algae biomass residue and lipid-rich fat, oil, and grease  
5 waste resulted in a specific methane production rate of 540 mL CH<sub>4</sub>/gVS<sub>influent</sub>·d with regards  
6 to a rate of 150 mL CH<sub>4</sub>/gVS<sub>influent</sub>·d when microalgae biomass was digested alone.<sup>44</sup>  
7 The co-digestion of *Chlorella* residues with glycerol, produced from the transesterification  
8 process of biodiesel production, was also examined by Ehiment et al. (2009)<sup>45</sup>. These authors  
9 showed the effect of the type of solvent used in the oil extraction step on methane yield. In  
10 particular, extraction solvents such as chloroform resulted in a repression of methane  
11 production. Therefore, where energy generation via anaerobic digestion of microalgae  
12 residues is planned, , investigations on possible solvent interferences on the microbial process  
13 should be performed before solvent selection. Nonetheless, the solvent inhibitory effects can  
14 be reduced by a rinsing step to remove the toxic solvent from biomass. In counterpart, the  
15 rinsing process may have important water and energy requirements and could evacuate  
16 unbound energy-rich polar molecules, thus reducing the calorific value of the biomass  
17 feedstock.<sup>45</sup>  
18 The information available in literature on this subject is still scarce and more investigation is  
19 needed to improve knowledge in this interesting option of microalgae biorefinery.

## 20 ***3.2 Products from the anaerobic digestion***

### 21 *3.2.1 Biogas*

22 The biogas produced by anaerobic digestion is characterized by a methane percentage  
23 between 60% and 70%, depending of the substrate characteristics.<sup>46</sup>  
24 A number of different pretreatments (thermal, chemical, enzymatic and mechanical  
25 pretreatments) have already proved their efficiency to enhance the methane yields.<sup>30</sup> For

1 instance, Passos et al. (2013)<sup>47</sup> detected an increment of the methane yield of 4%, 53% and  
2 62% when a temperature pretreatment of 55, 75 and 95°C was applied, respectively.  
3 Similarly, in BMP tests, microwave pretreatment showed an increase of microalgae solubility,  
4 leading to a final yield improvement from 12 % up to 78% depending on the power applied  
5 (from 300 to 900 W).<sup>48</sup>

6 Some other options, such as an increase in the lipid content, were also proposed to improve  
7 the methane yield. However, cultivation strategies (i.e. high light intensity, nutrient  
8 starvation) which would raise lipid accumulation in cells, would probably affect the overall  
9 microalgae biomass productivity. It is thus not yet clear whether a particular cultivation  
10 strategy would be favorable to further increase the methane yields. In spite of recent  
11 developments in the field of biomethane production from microalgae, an optimal scenario  
12 combining ease of cultivation, high biomass yields and high anaerobic biodegradability has  
13 still to be determined.

14 Furthermore, several operational strategies were recently tested to improve the methane  
15 potentials of microalgal biomass. Zamalloa et al. (2012)<sup>49</sup> employed a hybrid flow-through  
16 reactor (combining a sludge blanket and a carrier bed) to increase the retention time of the  
17 algae biomass and decouple hydraulic and solid retention times. Markou et al. (2013)<sup>50</sup>  
18 proposed an increase in biomass carbohydrates through a phosphorus limitation process as an  
19 attractive technique to improve the bio-methane yield. Indeed, these authors tested various  
20 percentages of carbohydrates in cells and observed a methane yield ranging between 123 and  
21 203 mLCH<sub>4</sub>/gCOD<sub>influent</sub> (chemical oxygen demand) corresponding to 20% and 60%  
22 carbohydrates, respectively.

23 Concerning biogas quality, an important factor affecting CH<sub>4</sub> proportion in the biogas is the  
24 pH, which controls the speciation of the carbonate system and the release of CO<sub>2</sub>. Rates and  
25 yields of CH<sub>4</sub> formation also often increase with digestion temperature.<sup>22</sup> However, since

1 microalgae hardly contain sulphurated amino acids (Becker, 2007)<sup>51</sup>, their digestion releases a  
2 lower amount of hydrogen sulfide than other types of organic substrates.

3

4 Biogas could thus be reused for microalgae growth, promoting the interesting possibility to  
5 close the flux of products and effluents. In fact, the exploitation of biogas energy within a co-  
6 generation process can produce a gas mixture mainly composed of CO<sub>2</sub> with the same quality  
7 as turbine gas. A comparison between flue gas from turbines, water heaters and ovens,  
8 refinery activities, coal ovens and fuel injection, reveals that the turbine gas composition is  
9 characterized by the lowest concentrations in toxic compounds (NO<sub>x</sub>, SO<sub>x</sub>, C<sub>x</sub>H<sub>y</sub>, CO, heavy  
10 metals and particles). Thus, the product resulting from biogas combustion can be a suitable  
11 source of inorganic carbon for microalgal cultures with low concentrations of toxic  
12 compounds. Moreover, the oxidized form of nitrogen and sulfur present in high  
13 concentrations in flue gas can contribute to fulfill microalgae nutrient requirements.

14 It is known that microalgae incorporate inorganic carbon as a primary nutrient, and not  
15 limiting carbon conditions is one of the key conditions to optimize microalgal production. On  
16 average, algae consume 1.83 g CO<sub>2</sub> to produce 1 g of biomass.<sup>12</sup> Thus, biological CO<sub>2</sub>  
17 fixation by microalgae is considered to be a promising mean for fixing CO<sub>2</sub>, combining  
18 environmental and economic advantages, by contributing to prevent global warming on one  
19 hand and supplying carbon for microalgae for the other hand.

20 Moreover, even though CO<sub>2</sub> fixation is often mentioned in literature, an accurate CO<sub>2</sub> mass  
21 balance taking into account the final biomass disposal is necessary to determinate the  
22 environmental impact of the overall process. In the case of fuel generation, the biomass  
23 originates from atmospheric CO<sub>2</sub> and will be ultimately converted back into CO<sub>2</sub> when the  
24 fuel is burned and, in this case, the process could be considered as carbon neutral rather than a



1 carbon sink. More discussion about the environmental impact of biofuel products generated  
2 by microalgae can be found in Lardon et al., (2009).<sup>52</sup>»  
3 CO<sub>2</sub> consumption rates reported in literature in bubbled columns reactors varied between 0.2  
4 and 27 g/m<sup>2</sup>·d, depending on the microalgae culture and operational conditions.<sup>53</sup> Traviesco et  
5 al. (1993)<sup>54</sup> as well as Doušková et al., (2009)<sup>55</sup>, fed microalgae with biogas produced by  
6 anaerobic fermentation of a sugar cane distillery stillage. They observed that algae were able  
7 to consume CO<sub>2</sub> directly from biogas as well as from other sources in a range of  
8 concentrations between 2% (v/v) and 56% (v/v) of CO<sub>2</sub> in the mixture. Moreover, Park and  
9 Craggs (2011a; 2011b)<sup>56,57</sup> showed an increase in algal/bacterial production by about 30%,  
10 concomitantly to a significant nutrient removal enhancement due to CO<sub>2</sub> addition. A  
11 supplement in CO<sub>2</sub> can also maintain the pH at a suitable value (usually 8), thus preventing  
12 inhibition of algal growth by ammonia.<sup>58</sup> Furthermore, a pH less than 8 can reduce nitrogen  
13 removal by physicochemical processes such as ammonia volatilization, and may increase  
14 algal nutrient assimilation.  
15 These facts highlight the large adaptability of microalgae to different substrates, which is an  
16 important added value for a microalgae-based biorefinery. Indeed, microalgae culture can be  
17 coupled to a number of industrial chains for low cost wastewater treatment and generation of  
18 bioproducts.

### 19 3.2.2 Digestate (liquid and solid phase)

20 Besides biogas, anaerobic digestion processes generate liquid and solid phase effluents  
21 (digestate) that are rich in phosphorus and organic nitrogen compounds, ideal for use as  
22 organic fertilizer. Within the management process of this product (direct spreading, drying,  
23 liming) the separation between solid and liquid phases is suitable for an optimal exploitation  
24 of the different components. Many options for nutrient extraction from the digestate are  
25 nowadays explored in order to produce high quality fertilizers (e.g. ammonia stripping for

1 ammonium sulfate production and phosphorus precipitation through struvite formation). The  
2 separation process, that can be improved by addition of organic or mineral flocculants,  
3 produces a liquid fraction, rich in mineralized elements that can be directly spread or  
4 precipitated (e.g. struvite) (Türker et Celen, 2007)<sup>59</sup> and a solid fraction, usually composted,  
5 dried and/or exploited as an organic supplement.<sup>560</sup>

6 The different forms of digestate are characterized by different bio availabilities. Some  
7 components are absorbed on the organic fraction of suspended solids. This absorption is a  
8 function of the chemical properties of the components and the physico chemical properties of  
9 the solids. Generally, 40 to 86% of the organic matter is present in the solid fraction (Moller,  
10 2012)<sup>61</sup> while the liquid phase is characterized by a low organic matter content. The solid  
11 fraction contains about 75% of phosphorus, which is directly absorbed or trapped with  
12 calcium, magnesium and nitrogen.<sup>61</sup> Similarly, complex reactions are responsible for the  
13 distribution of microelements in liquid or solid phase after the post-treatment. For example,  
14 with liquid swine manure, copper, zinc and manganese were absorbed on the smaller particles  
15 (between 1 and 60  $\mu\text{m}$ ) and were preferably mobilized in the liquid phase after separation.<sup>72</sup>

16 On the other hand, the recycling of nutrients from wastewater highlights the need for the  
17 characterization of the quality of the digestate, with special attention to pathogens and heavy  
18 metal concentrations. Although anaerobic digestion is classified as a process that significantly  
19 reduces pathogens, their elimination strictly depends on the microbial species, digester  
20 temperature and retention time.<sup>63</sup> Likewise, pH, anaerobic conditions, nitrogen and volatile  
21 fatty acids can affect some pathogens.<sup>63</sup> However, information about this aspect is still scarce,  
22 and evidence from literature points out the necessity to consider the variability of the digestate  
23 composition and the concentrations in pathogens and heavy metal as important factors.  
24 Therefore, further efforts are required to determine the operating conditions able to enhance

1 fertilizer properties and pathogen reduction, as well as to promote the digestate nutrient  
2 recycling.

3 The use of digestate as substrate for microalgae growth is particularly interesting for the  
4 reduction of the process inputs in a biorefinery concept coupling wastewater treatment,  
5 microalgae culture and anaerobic digestion. Indeed the outlet of the anaerobic digesters fed  
6 with microalgae or other biomass contains about 50% of the initial nitrogen that can be  
7 reused as a source of nutrients and water for microalgae growth.

8 In a context of nutrient recycling, the liquid phase of the digestate was tested as a possible  
9 source of nitrogen for algae cultivation. In fact, the digestate liquid is characterized by low  
10 organic matter and phosphorus concentrations, counterbalanced by high potassium and  
11 nitrogen concentrations (up to 80% in the form of ammonium) (Table 3). Moreover, the  
12 micro-element composition of digestates (Table 4) can cover the nutrient requirements of a  
13 microalgae population.<sup>66</sup>

14 Many studies report the use of digestate from urban wastewater treatment, manure, abattoir  
15 residue or swine slurry for microalgal growth.<sup>63,64,65,66,67</sup> Bchir et al, (2011)<sup>70</sup> obtained a high  
16 biomass production of  $5.29 \cdot 10^6$  cell/mL associated with an important content of chlorophyll  
17 (65.32 mg/L) after 42 days of culture of *Spongiochloris* sp fed with abattoir digestate. Chen et  
18 al. (2012)<sup>72</sup> tested a long-term cultivation of freshwater algae in anaerobic digested manure  
19 effluents and indicated that *Chlorella* and *Scenedesmus* were able to grow in high nutrient  
20 loads (40, 100 and 200 g/L TN). However, Bjornsson et al. (2013)<sup>73</sup> show a magnesium  
21 limitation in *Scenedesmus* sp. growth with liquid swine manure digestate.

22 A few studies also tested the digestate of microalgal biomass as substrate for microalgal  
23 growth. Doušková et al., (2009)<sup>55</sup> tested a pilot scale reactor for biogas production and  
24 subsequent microalgae cultivation. The process consisted of a 50 L mesophilic reactor fed in  
25 semi-continuous mode with pure stillage. The reactor was followed by a photobioreactor

1 constituted by a set of glass bubbled columns in a thermostatic bath continuously illuminated.

2 These researchers determined experimentally that the growth rates of microalgae grown on

3 digestate were similar to those obtained with urea as substrate (16gDW/L).

4 Several experiments also pointed out the existence of inhibitory effects on microalgal growth,

5 especially with manure wastewater or digestate as substrate (Table 5). Among the observed

6 effects, high ammonia concentrations were often responsible of microalgal growth

7 inhibition.<sup>74,80</sup> Indeed, although ammonia can be an excellent source of nitrogen for

8 microalgal growth, free ammonia is toxic for most strains of microalgae due to its uncoupling

9 effect on photosynthetic processes in isolated chloroplasts.<sup>81</sup>

10 Another cause of microalgae growth inhibition is light limitation mainly due to mutual

11 shading caused by a high biomass density.<sup>67,82,83</sup> No particular effect of digestate turbidity on

12 microalgal growth has yet been reported in literature. However, it should be noticed that the

13 digestate is diluted in almost all the experiments reported in literature.<sup>51,66,63</sup>

14 Nevertheless, once the inhibitory factors have been identified, their effect can be easily

15 overcome by substrate dilution or carbon dioxide addition (for pH and ammonia

16 concentration control) or, in the case of self-shading, a periodical harvesting could prevent

17 high microalgal concentrations.<sup>67</sup> In this sense, Cho et al. (2013)<sup>84</sup> used urban wastewater for

18 microalgae growth, by testing 1) the effluent from a primary settling tank, 2) the effluent from

19 an anaerobic digestion tank and 3) a digestate dilution. According to their results, *Chlorella*

20 sp. showed the highest biomass production (3.01 g dry cell weight/L) when digestate was

21 diluted with wastewater rejected from a sludge concentrate tank (10:90, v/v).

22 It should also be taken into account that, depending on the digester performance, digestate

23 may contain volatile fatty acids and microorganisms already present in the substrate or

24 produced by the anaerobic flora. Similarly, in the liquid phase, it is possible to observe

25 residue from the flocculation processes used for solid/liquid separation.

1 Thus, the variability of digestate composition has an important potential impact that has not  
2 yet been carefully studied.

### 3 ***3.3 Anaerobic digestion in microalgae-based biorefinery***

4 During the past recent years, different applications of microalgae anaerobic digestion have  
5 been integrated in a biorefinery concept moving the role of anaerobic digestion from a waste  
6 treatment to an organic matter conversion unit. Razon (2012)<sup>85</sup> proposed a process in which  
7 ammonia sulfate from the digestate is stripped, converting the ammonia to a solid form. Thus,  
8 it can be easily separated by gravity settling and processed into crystals further used as  
9 fertilizer, while the liquid part (~70%) can be used in agriculture or returned to the algal  
10 culture.

11 With similar objectives, De Schamphelaire and Verstate (2009)<sup>86</sup> proposed a closed loop  
12 system integrating an algal growth unit for biomass production, an anaerobic digestion unit to  
13 convert the biomass to biogas and a microbial fuel cell to treat further the effluent of the  
14 digester and produce electricity. To close the loop, nutrients from the digester are returned to  
15 the algal growth unit.

16 A recent study<sup>87</sup> investigated the selection of methanotrophic bacteria to produce  
17 polyhydroxybutyrate (PHB), which is a biodegradable polyester. In this case, biogas was used  
18 to feed microalgae and to stimulate methanotroph bacteria. Moreover, these researchers found  
19 that the symbiotic cooperation between microalgae and methanotroph bacteria led to the  
20 formation of harvestable bioflocs.

21 These studies show that it is possible to develop new interesting solution to integrate  
22 anaerobic digestion into a biorefinery concept. In this perspective, it is advisable to integrate  
23 different processes in order to generate new valuable products maximizing overall efficiency,  
24 while reducing operating costs and environmental impacts. To do this, multidisciplinary

1 research on systems biology, strain development, systems design, modeling and biorefining is  
2 required.

### 3 ***3.4 Economic and environmental aspects***

4 In spite of the increasing interest in anaerobic digestion of microalgae, little information on  
5 the economic aspects of this process is available in literature. Delrue et al (2012)<sup>88</sup> carried out  
6 an economic study of biodiesel production from microalgae considering anaerobic digestion  
7 as a treatment of microalgae residue. According to this study, the price of 1 liter of biodiesel  
8 varies between 1.94 and 3.35 €. Among the major bottlenecks identified in this study, the  
9 cultivation steps and the downstream processes play an important role. This indicates that  
10 more efforts are needed in order to reduce cultivation costs, optimize microalgae productivity  
11 and improve technologies for biomass valorization. Overall, anaerobic digestion methane  
12 yield positively impacts the net energy ratio, contributing to 33% of the total energy  
13 production. A recent study on the potential of microalgae as feedstock for methane  
14 production<sup>85</sup> found a cost of energy in the order of magnitude of 0.087-0.170 €/kWh<sup>-1</sup>. This  
15 study considered the microalgae biomass cultivated in a 400 ha (4 km<sup>2</sup>) raceway pond with  
16 inputs of fresh water, nutrients and sunlight. The harvesting step consists on a settling stage  
17 with flocculants followed by a dissolved air flotation. Then an anaerobic process is carried out  
18 at 30°C and the water and nutrients from the pre-concentration and anaerobic digestion stage  
19 are recirculated and the CO<sub>2</sub> from the flue gas is used for algae cultivation.

20 However, the wide range of data available in literature makes difficult an economical  
21 comparison between processes and even between units of the same process. Moreover, the  
22 economic studies available are based on theoretical models; the availability of data from real  
23 and large scale plants would certainly help to get more reliable information about the  
24 economic viability of microalgae biorefinery. An accurate economic and environmental study  
25 is especially needed for the most recent biorefinery solutions presented above.

1 From an environmental point of view, only few studies on microalgae biorefinery and  
2 anaerobic digestion have been recently published.<sup>52,89,40,42</sup> Concerning the environmental  
3 impact, the study carried out by Lardon et al. (2009)<sup>52</sup> confirmed the potential of microalgae  
4 as an energy source but emphasized on the imperative necessity of decreasing the energy and  
5 fertilizer consumption. Collet et al. (2011)<sup>90</sup> pointed out the electricity consumption as the  
6 main source of impacts and suggested that improvement of the efficiency of the anaerobic  
7 process under controlled conditions could be a possible solution for decreasing process  
8 consumption. Benemann et al. (2012)<sup>91</sup> found that oil production from microalgae coupled  
9 with the anaerobic digestion of microalgae residue does not require fossil energy inputs and  
10 does not produce greenhouse gas emissions.

11

#### 12 **4. Perspectives and further research**

13 This paper has emphasized several crucial points of microalgae-based bioprocesses that need  
14 to be developed in order to upgrade the potential of microalgal anaerobic digestion and to find  
15 new renewable and carbon-neutral products and energy sources.

16 Firstly, challenges regarding microalgal culture need to be solved. In fact, in spite of the  
17 increasing interest and the number of studies conducted in this field, there are still problems  
18 related to the high building and operating costs, the difficulty in controlling and optimizing  
19 the culture conditions, contamination by bacteria or microalgae, predators, unstable light  
20 supply and weather changes.

21 The selection of the most valuable microalgae strains for anaerobic digestion still requires  
22 research efforts. In this context, the genetic improvement can be a tool to create microalgae  
23 strains with high productivity and high methane potential that could improve anaerobic  
24 digestion efficiency.

1 Anaerobic digestion effectiveness could also be enhanced by the study and implementation of  
2 innovative pretreatments or co-digestion processes as well as reactor configurations and  
3 operation strategies.

4 Another bottleneck is the harvesting process, which is a crucial step for biomass production  
5 with low costs and low energy requirements.

6 Moreover, the benefit in closing the loop of microalgae biorefinery would require the  
7 extension of the actual limited knowledge on digestion of algal biomass residue. Another  
8 interesting aspect that deserves further attention is the quality of digestate and its properties as  
9 a substrate for microalgae growth and/or as fertilizer.

10 We report here some example of process coupling; however more biorefinery configurations  
11 incorporating a whole range of different installations should be further explored. In this  
12 context, a number of industries could combine their material flows in order to reach a  
13 complete utilization of all biomass components. In this way the residue from one industry  
14 (e.g. lignin from a lignocellulosic ethanol production plant) could become an input for other  
15 types of industry.

16 In line with the promising results produced from laboratory studies, a scaling-up of the  
17 technology from the laboratory to the pilot plant has now become essential in order to verify  
18 the sustainability of the process.

19 Finally, the increasing interest in developing industrial-scale microalgae-to-biofuel  
20 technology requires a detailed assessment of the costs and the potential environmental  
21 impacts of the entire process chain, from biomass production to the biofuel combustion.

22 Almost all environmental and economic assessments found in literature have been indeed  
23 based on assumptions and extrapolations from laboratory experiments and small-scale  
24 outdoor systems. Last but not least, the emissions of major greenhouse gasses (e.g. nitrous



1 dioxide and methane) during the microalgae cultivation stage have been ignored and real data  
2 remain necessary to improve life cycle assessment.

3

#### 4 **5. References**

- 5 1. Cherubini F. The biorefinery concept: Using biomass instead of oil for producing energy  
6 and chemicals. *Energ Convers Manag* **51**: 1412–1421 (2010).
- 7 2. Singh J and Gu S. Commercialization potential of microalgae for biofuels production. *Sust*  
8 *Energy Rev* **14**: 2596–610 (2010).3. Dismukes GC, Carrieri D, Bennette N, Ananyev GM and  
9 Posewitz MC . Aquatic phototrophs: efficient alternatives to land-based crops for biofuels.  
10 *Curr Opin Biotechnol* **19**: 235–240 (2008).
- 11 4. Becker EW. Microalgae Biotechnology and microbiology. Cambridge Press University  
12 293p, (1994).5. Williams PJLB and Laurens LML. Microalgae as biodiesel & biomass  
13 feedstocks: Review & analysis of the biochemistry, energetics & economics. *Energ Env Sci* **3**:  
14 554-590 (2010).
- 15 6. Harun R, Singh M, Forde Gareth M and Danquah MK. Bioprocess engineering of  
16 microalgae to produce a variety of consumer products. *Renew Sust Ener Rev* **14**: 1037–1047  
17 (2010).
- 18 7. Belarbi EH, Molina E and Chisti Y. A process for high yield and scaleable recovery of high  
19 purity eicosapentaenoic acid esters from microalgae and fish oil. *Process Biochem* **35**: 951–  
20 969 (2000).
- 21 8. Skjånes K, Rebours C and Lindblad P. Potential for green microalgae to produce hydrogen,  
22 pharmaceuticals and other high value products in a combined process. *Critical Reviews*  
23 *Biotechnol* **33**: 172–215 (2013).

- 1 9. Mendesa RL, Nobrea BP, Cardoso MT, Pereirab PA and Palavrab AF. Supercritical  
2 carbon dioxide extraction of compounds with pharmaceutical importance from microalgae.  
3 *Inorganica Chimica Acta* **356**: 328–334 (2003).
- 4 10. Pieber S, Schober S and Mittelbach M. Pressurized fluid extraction of polyunsaturated  
5 fatty acids from the microalga *Nannochloropsis oculata*. *Biomass Bioenergy* **47**: 474–482  
6 (2012).
- 7 11. Dhargalkar VK and Verlecar XN. Southern Ocean seaweeds: a resource for  
8 exploration in food and drugs. *Aquacult* **287**:229–242 (2009).
- 9 12. Amaro HM, Guedes AC and Malcata FX. Advances and perspectives in using microalgae  
10 to produce biodiesel. *Appl Energ* **88**: 3402–3410 (2011).
- 11 13. Harun R, Davidson M, Doyle M, Gopiraj R, Danquah M and Forde G. Technoeconomic  
12 analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility.  
13 *Biomass Bioenerg* **35**: 741–747 (2011).
- 14 14. Wi SG, Kim HJ, Mahadevan SA, Yang DJ and Bae HJ. The potential value of the  
15 seaweed Ceylon moss (*Gelidium amansii*) as an alternative bioenergy resource. *Biores*  
16 *Technol* **100**: 6658-6660 (2009).
- 17 15. Singh NK and Dhar DW. Microalgae as second generation biofuel. A review *Agronomy*  
18 *for Sustainable Development* **31**:605-629 (2011).
- 19 16. Wijffels RH, Barbosa MJ, Eppin MHM. Microalgae for the production of bulk chemicals  
20 and biofuels. *Biofuel, Bioprod Biorefin.* **4**: 287–295 (2010).
- 21 17. Domozych DS, Ciancia M, Fangel JU, Mikkelsen MD, Ulvskov P and Willats WGT. The  
22 cell walls of green algae: a journey through evolution and diversity. *Frontiers Plant Sci.* **3**: 82  
23 (2012).

- 1 18. Richmond, Handbook of Microalgal Culture: Biotechnology and Applied Phycology.  
2 Blackwell Science, Oxford, OX, UK, Ames, Iowa, USA (2004).
- 3 19. Nguyen THM and Vu VH. Bioethanol production from marine algae biomass: prospect  
4 and troubles. *J. Viet. Env.* **3**: 25-29 (2012).
- 5 20. Harun R, Danquah MK and Forde G.M. Microalgal biomass as a fermentation feedstock  
6 for bioethanol production. *J Chem Technol Biotechnol.* **85**: 199–203 (2009).
- 7 21. Naim R, Muhammad SUR, Sheeraz M, Zia URa, Kisay L, Jong-In H. Current status,  
8 barriers and developments in biohydrogen production by microalgae. *Ren Sust Energ Rev* 22  
9 571–579 (2013).
- 10
- 11 22. Ferreira AF, Marques AC, Batista AP, Marques PASS, Gouveia L, Silva CM. Biological  
12 hydrogen production by *Anabaena* sp. e Yield, energy and CO<sub>2</sub> analysis including  
13 fermentative biomass recovery. *Int J Hydrog En* 37 179-190 (2012).
- 14 23. Meuser JE, Anayev G, Wittig LE, Kosourov S, Ghirardi ML, Seibert M, et al. Phenotypic  
15 diversity of hydrogen production in chlorophycean algae reflects distinct anaerobic  
16 metabolisms. *J Bacteriol* **142**: 21-30 (2009).
- 17 24. Yacoby I, Pochekailov S., Topotrik H, Ghirardi ML, King PW and Zhang S.  
18 Photosynthetic electron partitioning between [FeFe]- hydrogenase and ferredoxin:NADP<sup>+</sup>-  
19 oxidoreductase (FNR) enzymes in vitro. *Proc Natl Acad Sci* **108**: 9396-9401 (2011).
- 20 25. Hidayet A, Fikret K. Bio-hydrogen production by different operational modes of dark and  
21 photo-fermentation: An overview. *Int J Hydrog En* 36: 7443-7459 (2011).
- 22 26. Lakaniemi AM, Tuovinen OH and Puhakka JA. Anaerobic conversion of microalgal  
23 biomass to sustainable energy carriers - A review. *Bioresource Technol* **135**: 222–31. (2013).

- 1 27. Guo XM, Trably E, Latrille E, Carrère H, Steyer JP. Hydrogen production from  
2 agricultural waste by dark fermentation: A review. *Int J Hydrogen Energ* 35: 10660-10673  
3 (2010).
- 4 28. Cheng CL, Lo YC, Lee KS, Lee DJ, Lin CJ, Chang JS. Biohydrogen production from  
5 lignocellulosic feedstock. *Biores Technol* 102: 8514–8523(2011).
- 6 29. Yang ZM, Guo RB, Xu XH, Fan XL, Li XP, Enhanced hydrogen production from lipid-  
7 extracted microalgal biomass residues through pretreatment. *Int. J. Hyd Energ* 35: 9618–9623  
8 (2010).
- 9 30. González-Fernández C, Sialve B, Bernet N and Steyer JP. Impact of microalgae  
10 characteristics on their conversion to biofuel. Part II: Focus on biomethane production.  
11 *Biofuel, Bioprod Biorefin* **6**: 205–18 (2012).
- 12 31. Mussnug LH, Klassen V, Schlüter A, Kruse O. Microalgae as substrates for fermentative  
13 biogas production in a combined biorefinery concept. *J Biotechnol.* **150**: 51–56 (2010).
- 14 32. Sialve B, Bernet N, and Bernard O. Anaerobic digestion of microalgae as a necessary step  
15 to make microalgal biodiesel sustainable. *Biotechnol Adv* **27**:409–416 (2009).
- 16 33. Frigon JC, Matteau-Lebrun F, Abdou RH, McGinn PJ, O’Leary SJB and Guiot SR.  
17 Screening microalgae strains for their productivity in methane following anaerobic digestion.  
18 *Appl Energ* **108**:100-107, (2013).
- 19 34. Chen Y, Cheng JJ and Creamer KS. Inhibition of anaerobic digestion process: a review.  
20 *Biores Technol* **99**: 4044-4064 (2008).
- 21 35. Olguín EJ. The cleaner production strategy applied to animal production. In: Olguín EJ,  
22 Sánchez G, Hernández E, editors. Environmental biotechnology an cleaner bioprocesses.  
23 London: Taylor and Francis. p. 227-243 (2000).

- 1 36. Phang SM, Miah MS Yeoh BG and Hashim MA. Spirulina cultivation in digested sago  
2 starch factory wastewater. *J Appl Phycol* **12**: 395-400 (2000).
- 3 37. Kayhanian M and Tchobanoglous G. Computation of C/N ratios for various organic  
4 fractions. *Biocycle* **33**:58-60 (1992).
- 5 38. Yen Hw and Brune DE. Anaerobic co-digestion of algal sludge and waste paper to  
6 produce methane. *Bioresource Technol* **98**:130–134 (2007).
- 7 39. González-Fernández C, Molinuevo-Salces B and García-González MC, Evaluation of  
8 anaerobic codigestion of microalgal biomass and swine manure via response surface  
9 methodology. *App. Energ* **88**:3448–3453 (2011b).
- 10 40. Subhadra BG and Edwards M. Coproduct market analysis and water footprint of  
11 simulated commercial algal biorefineries. *Appl. Energ* **88**:3515–3523 (2011).
- 12 41. Chisti Y. Biodiesel from microalgae. *Biotechnol Adv* **25**: 294–306 (2007).
- 13 42. Chisti Y. Biodiesel from microalgae beats bioethanol. *Trends Biotechnol* **26**:126-131  
14 (2008).
- 15 43. Yang Z, Guo R, Xu X, Fan X and Luo S. Hydrogen and methane production from lipid-  
16 extracted microalgal biomass residues. *Int J Hydrogen Energ* **36**:3465–3470 (2011).
- 17 44. Park S and Li Y. Evaluation of methane production and macronutrient degradation in the  
18 anaerobic co-digestion of algae biomass residue and lipid waste. *Bioresource Technol.*  
19 **111**:42–48 (2012).
- 20 45. Ehimen EA, Connaughton S, Sun Z and Carrington GC. Energy recovery from lipid  
21 extracted, transesterified and glycerol codigested microalgae biomass. *GCB Bioenergy* **1**:71-  
22 381 (2009).

- 1 46. Coombs J. The present and future of anaerobic digestion. In: Anaerobic Digestion: a  
2 Waste Treatment Technology. Elsevier Science Publisher LTD, Essex, 229 p. (1991).
- 3 47. Passos F, Garcia J and Ferrer I. Impact of low temperature pretreatment on the anaerobic  
4 digestion of microalgal biomass. *Biores technol* **138**:79-86.
- 5 48. Passos F, Garcia J and Ferrer I. Biogas production from microalgae grown in wastewater:  
6 Effect of microwave pretreatment. *Appl energ* **108**:168-175.
- 7 49. Zamalloa C, Boon N. and Verstraete W. Anaerobic digestibility of *Scenedesmus obliquus*  
8 and *Phaeodactylum tricornutum* under mesophilic and thermophilic conditions. *Appl Energ*,  
9 **92**:733–738 (2012).
- 10 50. Markou G, Angelidaki I and Georgakakis D. Carbohydrate-enriched cyanobacterial  
11 biomass as feedstock for bio-methane production through anaerobic digestion. *Fuel*, (April).  
12 doi:10.1016/j.fuel.2013.04.013 (2013).
- 13 51. Becker EW. Micro-algae as a source of protein. *Biotechnol Adv* **25**:207-210 (2007).
- 14 52. Lardon L, Helias A, Sialve B, Steyer JP and Bernard, O. Lifecycle assessment of biodiesel  
15 production from microalgae. *Environ Sci Technol* **43**:6475–6481 (2009).
- 16 53. Pires LCM, Alvim-Ferraz MCM, Martins FG and Simões M. Carbon dioxide capture  
17 from flue gases using microalgae: Engineering aspects and biorefinery concept. *Renew Sust*  
18 *Energ Rev* **16**: 3043–3053 (2012).
- 19 54. Travieso L, Sanchez EP, Benitez F. and Conde JL. *Arthrospira* sp. intensive cultures for  
20 food and biogas purification. *Biotechnol Letts* **15**: 1091-1094 (1993).
- 21 55. Doušková I, Kaštánek F, Maléterová Y, Kaštánek P, Doucha J and Zachleder V.  
22 Utilization of distillery stillage for energy generation and concurrent production of valuable

- 1 microalgal biomass in the sequence: Biogas-cogeneration-microalgae-products. *Energ*  
2 *Convers Manag* **51**:606-611 (2010).
- 3 56. Park JBK and Craggs RJ Nutrient removal in wastewater treatment high rate algal ponds  
4 with carbon dioxide addition. *Water Sci. Technol* **63**:1758-1764 (2011a).
- 5 57. Park JBK and Craggs RJ. Algal production in wastewater treatment high rate algal ponds  
6 for potential biofuel use. *Water Sci. Technol* **63**:2403-2410 (2011b).
- 7 58. Azov Y and Goldman JC. Free Ammonia Inhibition of Algal Photosynthesis in Intensive  
8 cultures. *Appl Environ Microbiol* **43**:735–739 (1982).
- 9 59. Türker M and Çelen I. Removal of ammonia as struvite from anaerobic digester effluents  
10 and recycling of magnesium and phosphate. *Biores Technol* **98**:1529-1534 (2007).
- 11 60. Al Seadi T and Lukerhurst C. Quality management of digestate from biogas plants used as  
12 fertiliser. IEA Bioenergy. Task 37- Energy from Biogas (2012).
- 13 61. Möller K. and Müller T. Effects of anaerobic digestion on digestate nutrient availability  
14 and crop growth: A review. *Eng Life Sci* **12**: 242–257. (2012).
- 15 62. Marcato CE, Pinelli E, Pouech P, Winterton P and Guisresse M. Particle size and metal  
16 distributions in anaerobically digested pig. *Bioresource Technol* **99**:2340-2348 (2008).
- 17 63. Godfree A and Farrell J. Processes for managing pathogens. *J. Environ. Qual.* **34**:105–  
18 113 (2005).
- 19 64. Sahlström L. A review of survival of pathogenic bacteria in organic waste used in biogas  
20 plants. *Biores Technol* **87**:161-166 (2003).
- 21 65. Cai T, Park SY and Li Y. Nutrient recovery from wastewater streams by microalgae:  
22 Status and prospects. *Renew Sust Energ Rev* **19**:360–369 (2013).

- 1 66. Wu JF and Pond WG. Amino acid composition and microbial contamination of *Spirulina*  
2 *maxima*, a blue-green alga, grown on the effluent of different fermented animal wastes.  
3 *Bulletin of Environmental Contamination and Toxicology*, **27**: 151–159 (1981).
- 4 67. Uggetti E, Sialve B, Latrille E, Steyer JP. Anaerobic digestate as substrate for microalgae  
5 culture: the role of ammonium concentration on the microalgae productivity. *Biores technol*  
6 In press. <http://dx.doi.org/10.1016/j.biortech.2013.11.036>.
- 7 68. Molinuevo-Salces B, García-González MC and González-Fernández C. Performance  
8 comparison of two photobioreactors configurations (open and closed to the atmosphere)  
9 treating anaerobically degraded swine slurry. *Bioresource Technol* **101**:5144–5149 (2010).
- 10 69. González-Fernández C, Molinuevo-Salces B and García-González MC. Nitrogen  
11 transformations under different conditions in open ponds by means of microalgae-bacteria  
12 consortium treating pig slurry. *Bioresouce Technol* **102**:960–6 (2011a).
- 13 70. Bchir FS, Gannoun H, El Herry S and Hamdi M. Optimization of *Spongiochloris* sp.  
14 biomass production in the abattoir digestate. *Bioresource Technol* **102**:3869-3876 (2011).
- 15 71. Coats ER, Searcy E, Ferin K, Shrestha D, McDonald AG and Briones A. An integrated  
16 two-stage anaerobic digestion and biofuel production process to reduce life cycle GHG  
17 emissions from US dairies. *Biofuels, Bioprod Biorefin* DOI: 10.1002/bbb.1408 (2013).
- 18 72. Chen R, Li R, Deitz L, Liu Y, Stevenson RJ and Liao W. Freshwater algal cultivation  
19 with animal waste for nutrient removal and biomass production. *Biomass Bioenerg* **39**:128–  
20 138 (2012).
- 21 73. Bjornsson WJ, Nicol RW, Dickinson KE and McGinn, PJ. Anaerobic digestates are useful  
22 nutrient sources for microalgae cultivation: functional coupling of energy and biomass  
23 production. *Journal of Applied Phycology*. DOI 10.1007/s10811-012-9968-0 (2013).



- 1 74. Kallqvist T and Svenson A. Assessment of ammonia toxicity in tests with the microalga,  
2 *Nephroselmis pyriformis*, Chlorophyta. *Water Res.* **37**:477-484 (2003).
- 3 75. Ogbonna JC, Yoshizawa H and Tanaka, H. Treatment of high strength organic wastewater  
4 by a mixed culture of photosynthetic microorganisms. *J Applied Phycol*, **12**: 277–284 (2000).
- 5 76. Perez-Garcia O, Escalante FME, De-Bashan LE and Bashan Y. Heterotrophic cultures of  
6 microalgae: metabolism and potential products. *Water res*, **45**: 11–36 (2011).
- 7 77. Wu JT, Chiang YR, Huang WY and Jane WN. Cytotoxic effects of free fatty acids on  
8 phytoplankton algae and cyanobacteria. *Aquatic toxicol*, **80**: 338–345 (2006).
- 9 78. Monteiro CM, Castro PML and Malcata FX. Metal uptake by microalgae: underlying  
10 mechanisms and practical applications. *Biotechnol progress*, **28**: 299–311 (2012).
- 11 79. Muñoz R and Guieysse B. Algal–bacterial processes for the treatment of hazardous  
12 contaminants: A review. *Water Res*, **40**: 2799-2815 (2006).
- 13 80. Li Y, Horsman M, Wang B, Wu N and Lan CQ Effects of nitrogen sources on cell growth  
14 and lipid accumulation of green alga *Neochloris oleoabundans*. *Appl. Microbial. Biotechnol.*  
15 **81**:629-636 (2008).
- 16 81. Crofts AR, Uptake of ammonium ion by chloroplasts, and the mechanism of amine  
17 uncoupling. *Biochem. Biophys. Res. Comm.* **24**:127–134 (1966).
- 18 82. Guieysse B, Borde X, Munoz R, Hatti-Kaul R and Nugier-Chauvin C. Influence of the  
19 initial composition of algal bacterial microcosms on the degradation of salicylate in fed batch  
20 culture. *Biotechnol Lett* **24**: 531–538 (2002).
- 21 83. Yuan X, Kumar A, Sahu AK and Ergas SJ. Impact of ammonia concentration on *Spirulina*  
22 *platensis* growth in an airlift photobioreactor. *Biores Technol* **102**:3234–9 (2011).

- 1 84. Cho S, Lee N, Park S, Yu J, Luong TT, Oh YK. and Lee T. (2013) Microalgae cultivation  
2 for bioenergy production using wastewaters from a municipal WWTP as nutritional sources.  
3 *Bioresource Technol* **131**:515-520 (2013).
- 4 85. Razon LF. Life cycle energy and greenhouse gas profile of a process for the production of  
5 ammonium sulfate from nitrogen-fixing photosynthetic cyanobacteria. *Biores Technol*  
6 **107**:339-346 (2012).
- 7 86. De Schampelaire L and Verstraete W. Revival of the biological sunlight to biogas energy  
8 conversion system. *Biotechnol Bioeng* **103**:296–304 (2009).
- 9 87. Van der Ha D, Nachtergaele L, Kerckhof FM, Rameiyanti D, Bossier P et al. Conversion  
10 of Biogas to Bioproducts by Algae and Methane Oxidizing Bacteria. *Env Sci Technol*  
11 **46**:13425-13431 (2012).
- 12 88. Delruea F, Setiera PA, Sahutb C, Cournac L, Roubauda A, Peltierb G and Fromenta AK.  
13 An economic, sustainability, and energetic model of biodiesel production from microalgae.  
14 *Biores Technol* **111** :191-200 (2012).
- 15 89. Zamalloa C, Vulsteke E, Albrecht J, Verstraete W. The techno-economic potential of  
16 renewable energy through the anaerobic digestion of microalgae. *Biores Technol* **102**: 1149-  
17 1158 (2011).
- 18 90. Collet P, Hélias A, Lardon L, Ras M, Goy RA, and Steyer JP. Life-cycle assessment of  
19 microalgae culture coupled to biogas production. *Biores Technol* **102**: 207–214 (2011). 91.  
20 Benemann J, Woertz I and Lundquist T. Life Cycle Assessment for Microalgae Oil  
21 Production. *Disruptive Sci Technol* **1**: 68–78 (2012).

22

23

24

1

2

3

4

5

1

## 2 6. Tables and Figures

Table 1. Biomass and raw energy productivities of land-based plants and microalgae culture (adapted from Dismukes et al. 2008).<sup>3</sup>

	Biomass productivity (dry tons/ha·y)	Raw energy productivity (GJ/ha·y)*
Corn grain	7	120
Sugarcane	73-87	1230-1460
Woody biomass	10-22	
Mixed grasses	3.6-15	61-255
Rapa seeds	2.7	73
Microalgae <i>Tetraselmis suecica</i>	10-22	700-1550
Microalgae <i>Arthrospira (Spirulina)</i>	27, 60-70	550, 1230-1435

3 \* Assuming heat of combustion, theoretical maximum energy content

1

2

Table 2. Distribution of the biochemical fractioning of a microalgae cell.<sup>4</sup>

<b>Biochemical compartment</b>	<b>Function</b>	<b>Mass concentration (%)</b>
Proteins	Structure and metabolism	40-60
Lipids	Structure and energetic reservoir	5-60
Carbohydrates	Structure et energetic reservoir	8-30
Nucleic acids	Support, vector and regulator of the genetic information	5-10

3

1

Table 3. Comparison between total nitrogen and phosphorus concentrations (mg/L) for different effluents (adapted from Cai et al., 2013)<sup>64</sup>.

<b>Effluent</b>	<b>Origin</b>	<b>Total Nitrogen</b>	<b>Total Phosphorus</b>
Urban wastewater	-	15-90	5-20
Digestate	Dairy manure	125-3456	18-250
	Poultry manure	1380-1580	370-382
	Sewage sludge	427-467	134-321
	Food waste and dairy manure	1640-1885	296-302

2

Table 4. Comparison of macro and micro element concentrations (mg/L) from different digestates.<sup>65</sup>

<b>Element</b>	<b>Bovine manure</b>	<b>Activated sludge</b>	<b>Pig manure</b>	<b>Poultry manure</b>
K	116	12	366	592
Na	38	31	111	214
Mg	60	32	225	54
Ca	171	267	174	42
Fe	9.1	3	38	2.5
Cu	0.04	0.02	0.02	0.04
Zn	0.44	0.16	0.08	0.1
Co	0.02	0.12	0.09	0.12
Mn	0.12	0.26	1.15	0.1
Cr	0.002	0.012	0.05	0.047

1

Table 5. Potential effects of the liquid digestate phase to microalgal growth

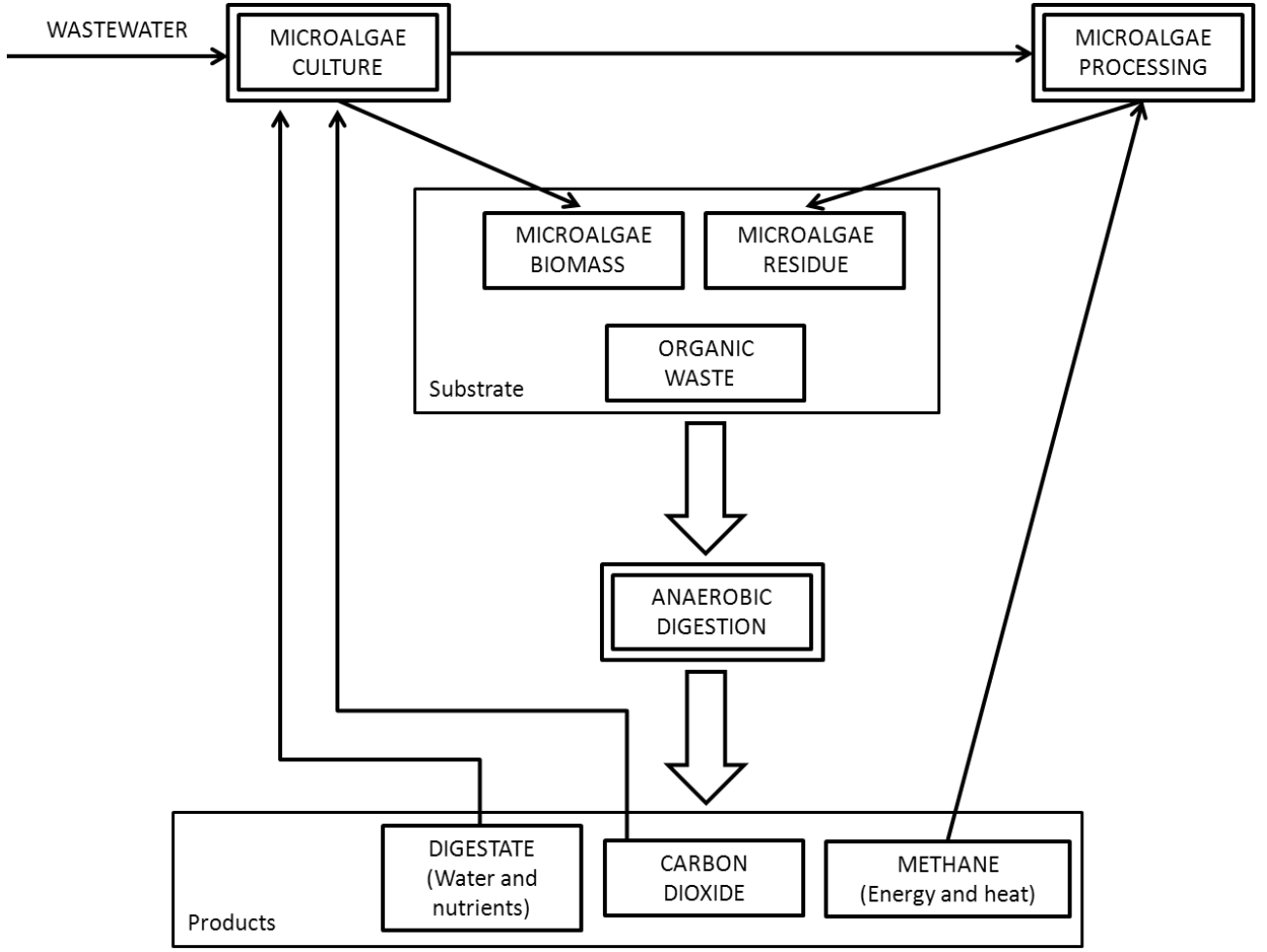
<b>Component</b>	<b>Potential effect</b>	<b>Reference</b>
Turbidity	Partial absorption of light energy	
Nitrogen concentration	Toxicity of the ammoniac form is pH is not regulated	74
		75
Volatile fatty acids concentration	Impact on the population equilibrium due to the stimulation of heterotrophic bacteria growth. Long chain fatty acids (>C14) can be toxic for some species.	76
		77
Flocculants	Coagulation effect leading to biomass sedimentation and performance limitation but also the bioavailability of essential nutrients such as phosphorus	
Microorganisms	Potential ecological impact (competition) and sanitary (depending on the microalgal exploitation industry)	
Heavy metals	Cellular toxicity, accumulation and potential sanitary impact (depending on the microalgal exploitation industry)	78
Organic trace elements	Potential cellular toxicity	79

2

3



1  
2



3  
4  
5  
6

Figure 1. Flux of materials in anaerobic digestion of microalgae biomass