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4 **Co-digestion strategies to enhance microalgae anaerobic digestion: A**
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7 **review**
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4 **Abstract**
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7 Microalgae biorefineries for the production of biofuels and high-value products have
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9 captured the attention of academia and industry. Implementing an anaerobic digestion step
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11 can enhance resource recovery from microalgae and microalgae residues. Anaerobic co-
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13 digestion, the simultaneous digestion of two or more substrates, is an opportunity to
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15 overcome the low biodegradability and the risk of ammonia inhibition associated with
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17 microalgae and microalgae residues mono-digestion. Besides, microalgae can also be used
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19 as co-substrate in biogas plants, with the aim of increasing the organic loading rate while
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21 providing alkalinity, macro- and micronutrients. Sewage sludge is the most researched co-
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23 substrate for microalgae since microalgae photobioreactors can be used for secondary,
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25 tertiary and anaerobic digestion supernatant treatment in wastewater treatment plants.
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27 However, microalgae and microalgae residues have been successfully co-digested with a
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29 wide variety of wastes, including crops, energy crops, paper waste, animal manure, vinasse,
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31 olive mill waste, and fat, oil and grease. Lipid-spent microalgae and glycerol co-digestion
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33 has also been largely researched due to the growing interest on microalgal-derived
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35 biodiesel. Most studies have assessed the impact of co-digestion on the methane yield and
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37 process kinetics through biochemical methane potential (BMP) tests. However, BMP test is
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39 not the most suitable method to assess the impact of co-digestion on other important factors
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41 such as supernatant nutrient content, digestate dewaterability, biosolids quality, and H₂S
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43 concentration in the biogas. Overall, more lab-scale and pilot-scale continuous experiments
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45 are needed to get a holistic understanding of microalgal anaerobic co-digestion.
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54 **Keywords:** biogas; anaerobic co-digestion; biorefinery; microalgal biomass; cyanobacteria;
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59 microalgae residues
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1. Introduction

The development of integrated microalgae-based facilities, so-called microalgae biorefineries, has attracted a substantial amount of attention from both academia and industry [1-3]. Microalgae biorefineries combine the production of biofuels (e.g. biodiesel, bioethanol, biogas) and high-value products (e.g. pigments, proteins, omega-3). Thus, microalgae biorefineries go one-step beyond the “third-generation biofuels” concept, which only aims at the production of liquid biofuels from microalgae. In biorefineries, microalgae cultivation costs can be reduced by using wastewater streams as nutrient source; achieving the dual goal of wastewater treatment and high-value chemicals production [4-6]. However, biosecurity regulations may restrict the application of microalgae products when using wastewater streams as source of nutrients [7-9].

Anaerobic digestion (AD), a biological process that transforms organic matter into renewable biogas energy, has been stated as the most suitable technology to maximize resource recovery from microalgae [10-13]. This technology is particularly suitable to treat the large amount of microalgae residues produced from the extraction of metabolites and reduce costs associated with microalgae residues treatment and disposal [2, 14-16]. Additional benefits of treating microalgae or microalgae residues via anaerobic digestion are the mobilization of nutrients (N and P) and the availability of CO₂, which can be recycled for microalgae cultivation [17-19]. However, microalgae AD is generally limited by its low methane potential (degradation extent) and conversion rate (degradation speed) as well as the risk of ammonia nitrogen inhibition.

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4 Pre-treatment methods, with or without co-products recovery, have been applied to disrupt
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6 microalgae cell wall, make their intracellular content more available and improve
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8 microalgae anaerobic biodegradability (extent and rate). Microalgae pre-treatments without
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10 co-products recovery have been reported to increase microalgae methane yield up to 100%
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12 [20, 21]. However, in most applications the increase in methane yield do not compensate
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14 the pre-treatment implementation and operational costs [22]. Indeed, the co-production of
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16 high-value chemicals and biogas has been identified as a more conceivable scenario than
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18 the production of biogas alone [3, 12, 23, 24]. Microalgae residues after co-product
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20 extraction have shown an increased anaerobic biodegradability when compared to raw
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22 microalgae, since the extraction step acts as a pre-treatment. For example, Ramos-Suarez
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24 and Carreras [14] observed an increase on *Scenedesmus* sp. methane yield from 140 to 272
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26 and 212 mLCH₄/gVS after the extraction of proteins and lipids respectively, while Parimi et
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28 al. [25] reported a methane yield increase from 181 to 254 mLCH₄/gVS for protein-spent
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30 *Spirulina platensis*. Even if upstream processing increases microalgae's anaerobic
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32 biodegradability, microalgae and microalgae residues are generally characterized by low
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34 methane yields (150-300 mLCH₄/gVS) and degradation rates (0.05-0.25 day⁻¹) when
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36 compared to common anaerobic digestion substrates, such as sewage sludge (200-350
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38 mLCH₄/gVS, 0.20-0.40 day⁻¹), animal manure (200-400 mLCH₄/gVS, 0.10-0.30 day⁻¹) and
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40 food waste (400-550 mLCH₄/gVS, 0.30-0.70 day⁻¹) [14, 26-35].
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53 Another key limitation for microalgae and microalgae residues anaerobic digestion is the
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55 risk of ammonia nitrogen inhibition, typically associated with microalgae's low carbon-to-
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57 nitrogen (C/N) ratio. Ammonia nitrogen is a potential inhibitor of the AD process that is
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59 released during the degradation of nitrogenous organic matter (e.g. proteins, amino acids,
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4 urea and nucleic acids) [36-38]. Indeed, microalgae biodegradability can be improved by
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6 different strategies, such as cultivating a different microalgae strain, tuning cultivation
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8 conditions, and using pre-treatments [22, 39, 40]. However, a high protein content and a
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10 low C/N ratio is common across all microalgae species. The risk of ammonia inhibition
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12 limits the maximum organic loading rate (OLR) at which microalgae digesters can be
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14 operated. An OLR around $2 \text{ gVS}/(\text{L}_r \cdot \text{day})$ has been observed as OLR threshold prior
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16 evidence of process inhibition [16, 41-44]. This low OLR threshold is a critical constraint
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18 for microalgae and microalgae residues anaerobic digestion feasibility, requiring (i) longer
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20 hydraulic retention times (HRT), i.e. larger digester volume or (ii) lower influent organic
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22 matter concentration. Either way, the resulting low volumetric methane yields
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24 ($\text{LCH}_4/(\text{L}_r \cdot \text{day})$) compromises the economic feasibility of microalgae AD.
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33 Anaerobic co-digestion (AcoD), the simultaneous digestion of two or more substrates, is a
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35 well-established option to overcome the drawbacks of mono-digestion and improve the
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37 economic feasibility of biogas plants. The latter is a result of the higher methane production
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39 and the treatment of several wastes in a single facility [45-48]. Microalgae and microalgae
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41 residues have been co-digested with a large variety of co-substrates, such as sewage sludge,
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43 animal manure, food waste, crops, glycerol, paper waste, and fat, oil and grease (FOG).
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45 Although the improvement of the methane production is mainly a consequence of the
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47 increased OLR rather than synergisms, ideal co-substrates for microalgae are highly
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49 biodegradable carbon-rich substrates, which boost methane production without increasing
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51 the nitrogen load [47]. Additionally, microalgae can be used as co-substrate in existing
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53 biogas plants. For instance, Schwede [49] explored the possibility of substituting pig
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4 manure by microalgae as source of alkalinity and macro- and micronutrients in corn silage
5 anaerobic digestion.
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11 Multiple microalgae anaerobic co-digestion mixtures and scenarios have been researched.

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13 Fig. 1 illustrates the most researched microalgae co-digestion scenarios in the literature,
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18 • High-value products biorefinery (Fig. 1A): high-value products are extracted from
19 microalgae and microalgae residues are co-digested with an external co-substrate
20 [14, 24, 25, 50]. In such scenario, CO₂ from biogas combustion can be recycled for
21 microalgae cultivation, while biosecurity regulations may restrict the use of the AD
22 supernatant (liquid fraction after digestate solid/liquid separation) for microalgae
23 cultivation.
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- 26 • Biodiesel biorefinery (Fig. 1B): lipid-spent microalgae is co-digested with glycerol,
27 by-product of lipids transesterification for biodiesel production [14, 16, 51, 52].
28 Anaerobic digestion supernatant and CO₂ from biogas combustion are recycled for
29 microalgae cultivation.
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- 32 • Secondary treatment in wastewater treatment plants (Fig. 1C): a high rate algal pond
33 (HRAP) is used for municipal wastewater treatment (instead of waste activated
34 sludge (WAS)) and harvested microalgae are co-digested with primary sludge [20,
35 53-58].
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- 38 • Tertiary treatment in wastewater treatment plants (Fig. 1D): a photobioreactor is
39 installed after the activated sludge unit to improve the quality of the final effluent
40 and harvested microalgae are co-digested with sewage sludge [12, 59, 60].
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- Anaerobic digestion supernatant treatment (Fig. 1E): a HRAP is used to remove nutrients from the anaerobic digestion supernatant and harvested microalgae are used as co-substrate. This approach have been studied to decrease the nutrient content of the return stream in wastewater treatment plants [6, 59, 61-64] and to improve the effluent quality of animal manure anaerobic digesters [9, 15, 50, 65-67].
 - Microalgae as co-substrate in an existing biogas plants (Fig. 1E): microalgae cultivated outside the biogas plant [49, 68-71] or collected from microalgae blooms [44, 72, 73] are added as co-substrate to an existing anaerobic digester.

The aim of this publication is to present a comprehensive and critical review about the recent achievements and perspectives of microalgae (including cyanobacteria) anaerobic co-digestion. The following sections discuss the anaerobic co-digestion of microalgae and microalgae residues with sewage sludge, animal manure, and a wide variety of agri-industrial wastes. Literature results are summarized in tables, where the methane yield improvement was calculated by comparing the experimental methane yield of the mixture with its theoretical methane yield. The review also identifies several knowledge gaps that warrant further investigation.

2. Co-digestion of microalgae and sewage sludge

Sewage sludge (mix of primary and waste activated sludge) is the most researched co-substrate for microalgae. The cultivation of microalgae in wastewater treatment plants (WWTP) has been used as an alternative to the conventional activated sludge reactor (Fig.

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4 1C), to polish the final effluent (Fig 1D) and to treat the anaerobic digestion supernatant
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6 (Fig. 1E) [7, 12, 56, 57, 64]. Additionally, microalgae ponds are a well-known technology
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8 for wastewater treatment [5, 74], which eases the adoption of microalgae cultivation
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10 systems in WWTP.
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16 On the one hand, the integration of microalgae cultivation as tertiary treatment and
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18 supernatant treatment aims to improve nutrients removal (N and P) from wastewater, while
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20 generating an additional co-substrate for sewage sludge. The cultivation of microalgae on
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22 anaerobic digestion supernatant is of special interest since it has the potential to (i) reduce
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24 the nutrient load of the return stream to the head of the plant, which represents up to 20% of
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26 the WWTP nutrient load, (ii) mitigate greenhouse gases emissions by using CO₂ from
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28 biogas or biogas combustion for microalgae growth, and (iii) generate significant amounts
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30 of microalgae as onsite co-substrate, which lowers the uncertainty about co-substrate
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32 availability and seasonality [47, 75]. Nonetheless, the supernatant may need to be pre-
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34 treated and/or diluted to reduce the presence of microalgae growth inhibitory compounds
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36 and improve light transmittance [7, 59, 76]. On the other hand, microalgae-based WWTPs,
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38 where HRAPs are used as secondary treatment, stand as a low-energy wastewater treatment
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40 system for regions with sufficient surface area and solar radiation [5, 56, 58]. In HRAP,
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42 microalgae grow in symbiosis with heterotrophic bacteria responsible of organic matter
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44 degradation; thus, the harvested biomass consists of a mix community of microalgae,
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46 bacteria and protozoa [77]. In this scenario, microalgae from the HRAP are co-digested
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48 with primary sludge from the primary settler.
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4 Microalgae and sewage sludge co-digestion is not a new concept, since the first published
5 study dates from 1983, when Samson and LeDuy [78] co-digested *Spirulina maxima* with
6 three different wastes, including sewage sludge. However, the number of papers dealing
7 with this topic has grown exponentially over the last years alongside the growing interest
8 on microalgal-derived biofuels. Most of these studies have been carried out using batch
9 assays, so-called biochemical methane potential (BMP) tests, at mesophilic conditions
10 (Table 1). Nevertheless, several studies have researched the performance of this mixture in
11 lab-scale continuous systems, such as continuous stirred tank reactors (CSTR) (Table 2).
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13 The main differences between those studies are related to the microalgae strain, sewage
14 sludge composition (primary and/or waste activated sludge) and the composition of the co-
15 digestion mixture.
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33 Most of the BMP-based studies analyzed a wide range of proportions between both co-
34 substrates. Beltrán et al. [79], Garoma and Nguyen [63], Lee et al. [80], Mahdy et al. [20],
35 and Neumann et al. [81] tested the co-digestion of different microalgae species and WAS at
36 25, 50 and 75 %. The same mixture range was tested by Mahdy et al. [20] and Solé-Bundó
37 et al. [53, 55] for primary sludge, by Caporgno et al. [82], Du et al. [83], and Olsson et al.
38 [62] for sewage sludge, and by Lu and Zhang [84] for septic sludge. Exploring a wide range
39 of proportions between microalgae and sludge is needed since the amount and
40 characteristics of both substrates would vary through the year depending on the wastewater
41 temperature and composition, as well as on the treatment plant design and operational
42 conditions [55, 57, 58]. In this regard, Passos et al. [56], who explored the feasibility of a
43 microalgae-based wastewater treatment plant (similar to Fig. 1C), calculated that the
44 proportion between microalgal biomass and primary sludge would be around 30/70% in
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4 winter and 60/40% in summer (VS-basis). Peng and Colosi [12], who performed a life
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6 cycle assessment on the implementation of a microalgae pond as tertiary treatment (similar
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8 to Fig. 1D), estimated that proportion between microalgae and sewage sludge would vary
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10 between 5/95% and 20/80% (VSS-basis). Therefore, mixtures where microalgae proportion
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12 represent less than 50% of the mixture may represent better WWTP scenarios. Peng and
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14 Colosi [12], Olsson et al. [62], Wang et al. [85], and Yuan et al. [59] studied mixtures with
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16 microalgae proportion below 50% (Table 1). Finally, Wagner et al. [86] studied the
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18 possibility of using bacterial biomass from an enhanced biological phosphorus removal
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20 system (similar to WAS) as bioflocculant for microalgae harvesting and subsequent
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22 anaerobic co-digestion. According to the authors, using 10 grams of bacterial biomass per
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24 gram of microalgae reduced the polymer dosing by 40%.
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33 BMP tests results show that the methane yield obtained from microalgae and sludge
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35 mixtures is proportional to the amount of microalgae and sludge. However, some authors
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37 have reported synergies (increased methane yield compared to the proportional one) of up
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39 to 25% [79, 85, 86]. Thorin et al. [48] noted that in most cases the improved methane yield
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41 could not be substantiated if the methane yield uncertainty was considered. Additionally, it
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43 should be considered that in full-scale plants minor methane yield improvements due to
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45 synergisms would be masked by natural variations of the substrates load, composition and
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47 biodegradability.
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55 Although microalgae and sludge co-digestion research has primarily focused on the
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57 methane yield, the feasibility of the process is also linked to the kinetics of the limiting step
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59 [87, 88]. As a highly particulate substrates, microalgae and sewage sludge anaerobic
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4 digestion is limited by the hydrolysis rate. The reported first-order constant rates for
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6 microalgae range between 0.03 and 0.24 day⁻¹ (average of 0.12 day⁻¹); which is at the lower
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8 end of the first-order constant rates reported for sewage sludge [28, 89]. With the exception
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10 of Wagner et al. [86], publications comparing the degradation kinetics of microalgae and
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12 sewage sludge mono-digestion and co-digestion observed a 20 – 50% improvement of the
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14 degradation kinetics under co-digestion conditions [34, 55, 79-81]. An improvement in
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16 degradation kinetics has also been reported when microalgae was co-digested with other
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18 substrates [14, 35, 90, 91]. The reasons behind the kinetic improvement under co-digestion
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20 conditions remain unexplored and warrants further research, since it opens the possibility to
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22 reduce treatment time or, if treatment time is maintained, improve waste stabilization.
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24 However, it should be noted that in BMP tests the apparent degradation kinetics are
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26 influenced by the inoculum capabilities [92, 93]. In this regard, Beltran et al. [79], Lee et al.
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28 [80], Olsson et al. [34], Solé-Bundó et al. [55], and Wagner et al. [86] used digested sewage
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30 sludge as inoculum, while Neumann et al. [81] used granular biomass from a UASB
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32 reactor. Digested sewage sludge is a suitable inoculum for this mixture. Moreover, it is the
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34 recommended inoculum by Holliger et al. [94] and Raposo et al. [30] when a fully adapted
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36 inoculum is not available. However, the correlation between the degradation kinetics
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38 observed in BMP tests and continuous reactors is a topic of current research and discussion
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40 within the anaerobic community.
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53 Despite the higher methane production, the implementation of anaerobic co-digestion in a
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55 WWTP has a direct impact on other key factors, such as the supernatant nutrient content,
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57 digestate dewaterability, biosolids quality and biogas composition (e.g. H₂S); all of them
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59 directly affecting the treatment costs [95-97]. The impact of a co-substrate on digestate
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4 dewaterability, biosolids stability and amount of residual solids to be handled (non-
5 biodegradable organic matter) are of particular importance since they affect the volume of
6 biosolids to be transported outside the WWTP as well as the digestate management
7 opportunities [34, 53, 59, 98].
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16 Regarding digestate dewaterability, Yuan et al. [59] reported that co-digesting 5 and 15%
17 of *Spirulina platensis* with WAS improved the digestate dewaterability when compared to
18 WAS alone. Nonetheless, in the same study, the digestate dewaterability worsened when 5
19 and 15% of *Chlorella* sp. were co-digested with WAS [59]. Conversely, Wang et al. [85]
20 reported that the anaerobic co-digestion of *Chlorella* sp. and WAS improved digestate
21 dewaterability at low *Chlorella* sp. proportions (4 and 11% weigh-basis), but worsened it at
22 a higher proportion (41% *Chlorella* sp.). However, these results should be interpreted
23 carefully since the dewaterability was measured on digestates obtained from BMP tests. In
24 a BMP test, the properties of the digestate are mostly controlled by the inoculum properties
25 rather than by substrates properties [42, 50]. In continuous lab-scale digesters, Solé-Bundó
26 et al. [53, 55] showed that the dewaterability from the digester treating a mix of primary
27 sludge and pre-treated microalgae (75/25% VS-basis) was better than the dewaterability
28 from the digester treating pre-treated microalgae and the digester treating non-pre-treated
29 microalgae. Olsson et al. [34] also reported an improvement in digestate dewaterability
30 when microalgae was added to sewage sludge (37/63% VS-basis). All previous studies
31 evaluated digestate dewaterability by determining the capillarity suction time (CST), likely
32 due to its simplicity and affordability. However, the CST is a proxy parameter for
33 dewaterability since it does not resemble the actual dewatering process and it fails to
34 predict the solids concentration of the dewatered cake [99]. Future research should
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4 complement CST with other dewaterability methods, such as thermo-gravimetric [100],
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6 filtration-centrifugation [101] and rheology analysis [99, 102, 103].
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11 Finally, the circular economy paradigm and the cradle-to-cradle concept require the
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13 development of auto-regenerative production systems, where waste products are converted
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15 into useful materials [95]. Therefore, beyond biogas production, AD plants need to find
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17 sustainable management and disposal solutions for the biosolids [104, 105]. Agricultural
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19 reuse is regarded as the best option to recycle the nutrients contained in the digestate [47,
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21 106]. However, this can only be done when the digestate quality fulfils the legal quality
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23 requirements. Solé-Bundó et al. [53], who assessed the digestate quality (i.e. concentration
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25 of nutrients, heavy metals, pathogens, phytotoxicity and organic matter stability),
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27 concluded that the digestate from primary sludge and microalgae co-digestion was more
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29 suitable for agricultural reuse than the digestate from microalgae mono-digestion [53].
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31 However, Olsson et al. [34] noted that using as co-substrate microalgae grown on flue gas
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33 as CO₂ source increased the heavy metal content in the digestate, making it unsuitable to be
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35 used as fertilizer. The authors related the higher heavy metal content in microalgae to the
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37 uptake of heavy metals from the flue gas and recommended to carefully assess the source
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39 of CO₂.
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50 **3. Co-digestion of microalgae and agri-industrial wastes**

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52 Agri-industries supply products to the food and fodder markets as well as a wide range of
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54 processing industries. Nonetheless, the production and processing of these products result
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56 in the generation of large amount of wastes [107]. AD stands out as a suitable technology to
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58 reduce the environmental impact of agri-industrial wastes and increase the energy self-
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4 sufficiency of these industries. However, agri-industrial wastes are characterized by a high
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6 C/N ratio, which can affect AD performance due to poor alkalinity and deficit of macro-
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8 and micronutrients [49, 108-110]. Co-digesting microalgae with agri-industrial wastes has
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10 been suggested as an option to overcome these limitations [47, 111]. Additionally,
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12 microalgae can be cultivated using marginal soil in regions where other suitable co-
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14 substrates are not available [49, 81]. Conversely, agri-industrial wastes can be used as co-
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16 substrates in microalgae digesters to increase the digester OLR and methane yield without
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18 increasing (or even diluting) the nitrogen concentration.
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26 Microalgae have been co-digested with a wide range of agri-industrial wastes, including
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28 crops (e.g. corn silage, corn stover, wheat straw), energy crops (e.g. switchgrass,
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30 *Pennisetum*), waste paper/sludge, olive mill waste, FOG, and glycerol. Most of the studies
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32 focused on improving AD performance by balancing the C/N ratio since agri-industrial
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34 wastes present relatively high C/N ratios (>45), while microalgae present relatively low
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36 C/N ratios (< 12). Table 3 and Table 4 summarize the studies co-digesting microalgae and
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38 agri-industrial waste in BMP tests and in continuous lab-scale reactors respectively.
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45 Fig. 2 illustrates the calculated improvement of the methane yield depending on the C/N
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47 ratio for the studies co-digesting microalgae and agri-industrial wastes. The methane yield
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49 improvement was calculated by comparing the experimental methane yield of the mixture
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51 with its theoretical methane yield. The latter was calculated by the product summation of
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53 each substrate methane yield in mono-digestion and their proportion in the mixture [79,
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55 112]. Positive values (>10%) indicate synergism (i.e. the mixture produces more methane
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57 than expected), while negative values (<10%) indicate antagonism (i.e. the mixture
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4 produces less methane than expected). Values between -10% and 10% were considered
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6 neutral (neither synergistic nor antagonistic) in order to account for the uncertainty around
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8 measured methane yields and the propagation of multifarious analytical errors. Most studies
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10 target mixtures with C/N ratios ranging between 15 and 30, which falls into the reported
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12 optimum range for optimum AD performance [16, 108, 111]. However, both neutral and
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14 synergistic responses are observed within this C/N range. Given the variability of
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16 improvement in methane yields for a certain C/N ratio, it is clear that optimizing co-
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18 digestion mixtures based on the C/N ratio is an oversimplification. The C/N ratio is a proxy
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20 for macronutrients availability, ammoniacal nitrogen concentration and system alkalinity.
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22 However, it does not consider other important factors, such as substrate biodegradability,
23
24 secondary risk of inhibition and micronutrients. Thus, the long legacy of using the C/N
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26 ratio as key factor to explain the synergisms and antagonisms occurring during anaerobic
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28 co-digestion has caused an overlook of the actual mechanisms behind such phenomena.
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38 Regarding the impact of the C/N on methane yield, Solé-Bundó et al. [91] observed that
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40 adding NH₄Cl to wheat straw did not increase its methane yield in BMP tests. One could
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42 argue that the impact of adding NH₄Cl was masked by the inoculum which in BMP testing
43
44 is typically the main source of macro- and micronutrients, alkalinity and microorganisms
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46 [92, 94]. However, a similar result was obtained by Yen and Brune [41] in a research study
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48 devoted to co-digest microalgae and waste paper in continuous reactors. Yen and Brune
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50 [41] observed that adding NH₄Cl to decrease the waste paper C/N ratio from 2000 to 21.5
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52 was not enough to explain the synergism occurring during microalgae and waste paper co-
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54 digestion. The authors hypothesized that microalgae improved waste paper anaerobic
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56 digestion by balancing the C/N ratio and providing a range of essential micronutrients.
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4 Herrmann et al. [42], who co-digested *Spirulina platensis* with three different carbon-rich
5 substrates (i.e. barley straw, beet silage, and brown seaweed) each in a separate CSTR, also
6 noted that the C/N ratio is not the only parameter to consider when optimizing co-digestion
7 mixtures. Besides the digester treating only *Spirulina platensis*, the other three CSTRs were
8 fed with the co-digestion mixture that provided a C/N ratio of 25 (i.e. 15% barley straw,
9 45% beet silage and 55% brown seaweed on a VS-basis). Herrmann et al. [42] reported that
10 the reactor digesting *Spirulina platensis* was inhibited (substantial decrease of the methane
11 yield) when the OLR was increased from 1 to 2 gVS/(L_r·day), whereas the CSTRs co-
12 digesting barley straw, beet silage and brown seaweed were inhibited when the OLR was
13 subsequently increased to 3, 4, and 5 gVS/(L_r·day) respectively. As the maximum OLR for
14 stable AD operation increased together with the co-substrate proportion, Herrmann et al.
15 [42] concluded that the difference in performance was linked to the occurrence of ammonia
16 inhibition rather than the C/N ratio itself.

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38 Synergisms associated with microalgae anaerobic co-digestion have also been linked to
39 other parameters more difficult to quantify and monitor than the C/N ratio, ammonia
40 concentration, and alkalinity. For instance, Schwede et al. [49] claimed that the
41 micronutrients (i.e. Co, Mo, Ni, Na) supplemented by *Nannochloropsis salina* were one of
42 the key factors preventing digestion failure when the OLR was increased from 2 up to 4.7
43 gVS/(L_r·day). Indeed, micronutrients (e.g. Co, Mo, Fe, Ni and Se) are well-known
44 cofactors in numerous enzymatic reactions involved in the biochemistry of methane
45 formation [109, 113]. Yen and Brune [41] results may also indicate that the observed
46 increase on cellulase activity (enzyme that catalyzes cellulose hydrolysis) was partly related
47 to the supplementation of micronutrients by microalgae. However, Zhong et al. [44] did not
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4 observe an improvement of cellulose activity when *Microcystis* sp. was co-digested with
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6 corn straw, as cellulase activity decreased as the corn straw proportion in the mixture
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8 decreased. The role of micronutrients and enzymes activity on anaerobic (co-)digestion
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10 performance warrants further research.
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16 Although most studies have emphasized possible synergisms between substrates, more
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18 attention should be given to inhibition/antagonism phenomena occurring during anaerobic
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20 co-digestion, since they are clear indicators of constraints associated with the co-digestion
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22 of a particular co-substrate. In practice, co-substrate selection and dose are primarily
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24 controlled by the availability and occurrence of secondary inhibition phenomena (e.g.
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26 salinity, heavy metals, ammoniacal nitrogen, volatile fatty acids (VFA), long chain fatty
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28 acid (LCFA), biogas H₂S concentration) [45, 47, 96, 97, 114-118]. For instance, the
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30 addition of microalgae into a digester could increase the heavy metals concentration in the
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32 digestion media, which may not only impact the AD performance but also the possibility of
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34 reusing the digestate on land [14, 34, 53]. In the same way, the addition of microalgae
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36 grown on brackish and brine water can increase the concentration of Na⁺ and other cations
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38 (e.g. Ca²⁺, K⁺ and Mg²⁺) in the digestion media, all of them well-known inhibitors of the
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40 AD process [116, 119]. Na⁺ and K⁺ concentrations may also be increased when crude
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42 glycerol, by-product of biodiesel production, is used as co-substrate in a microalgae
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44 digester; although the main limitation when using crude glycerol as co-substrate is linked to
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46 the accumulation of propionate [98]. Similarly, the risk of LCFA inhibition limits the dose
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48 of FOG as co-substrate [47, 115, 120]. Finally, it is worth highlighting that antagonisms
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50 occurring during co-digestion are more difficult to detect and quantify than synergisms.
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52 This is because (i) the impact of inhibitors and intermediate metabolites in BMP testing is
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4 diluted, and (ii) long operation time and a certain co-substrate loading rate may be required
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6 prior an inhibitor reaches its inhibitory concentrations.
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10 11 **4. Co-digestion of microalgae and animal manure** 12

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14 The life cycle assessment and energy analysis carried out by Wang et al. [67] and Zhang et
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16 al. [15] showed that treating animal manure anaerobic digestion supernatant with
17
18 microalgae ponds is an opportunity to reduce the environmental impacts associated with
19
20 manure management (e.g. eutrophication, global warming) and increase bioenergy
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22 production through co-digestion. The configuration analyzed in both studies (similar to Fig.
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24 1E) was found environmentally and energy superior to direct land application and manure
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26 anaerobic mono-digestion [15, 67]. Nonetheless, Zhang et al. [15] observed that the
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28 profitability of this scheme was highly dependent on the sale price of nutrient credits.
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36 Animal manure (i.e. pig, cattle, and poultry) and microalgae co-digestion has received less
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38 attention than microalgae co-digestion with sewage sludge (Section 2) or agri-industrial
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40 waste (Section 3). This is likely due to the relatively low C/N ratio of both substrates,
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42 which increases the risk of ammonia inhibition. However, the possibility of recovering
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44 nutrients, improving the effluent quality, and producing an onsite co-substrate through
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46 microalgae cultivation makes manure and microalgae co-digestion worth investigating.
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48 Even more when Mahdy et al. [66], who co-digested *Chlorella vulgaris* and cattle manure,
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50 showed that anaerobic biomass could be acclimated to tolerate free ammonia and total
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52 ammonical nitrogen (TAN) concentrations up to 650 mgNH₃-N/L and 3.8 gTAN/L
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4 Most of the animal manure and microalgae co-digestion research has been carried out in
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6 BMP tests, with pig manure being the most studied (Table 5). The BMP test is a suitable
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8 analytical method to understand the interaction between substrates occurring during co-
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10 digestion. However, a BMP test is not the most suitable method to assess the impact of
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12 inhibitors (e.g. free ammonia), since they are masked by the inoculum [42, 50]. Regarding
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14 substrates interaction, Astals et al. [50], Gonzalez-Fernandez et al. [65], Tsapekos et al.
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16 [71] and Wang et al. [67] observed that co-digesting microalgae with pig manure increased
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18 microalgae anaerobic biodegradability to different extent. An improvement of the methane
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20 yield (compared to the proportional one) was also obtained by Mahdy et al. [66] and
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22 Prajapati et al. [121] when co-digesting microalgae and cattle manure, and by Li et al. [9]
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24 and Meneses-Reyes et al. [122] when co-digesting microalgae and poultry manure (Table
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26 5). Mahdy et al. [66] and Prajapati et al. [121] attributed the synergistic effect to the
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28 improved C/N ratio, while Li et al. [9] attributed it to the N/P ratio. Although the C/N ratio
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30 is the most reported parameter to explain the synergies occurring during anaerobic co-
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32 digestion, synergism could not always be linked to the C/N ratio [50, 65, 70, 122]. In this
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34 regard, Astals et al [50] hypothesized that synergism was due to the addition of specific
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36 microbes from pig manure, since other factors previously used to explain co-digestion
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38 synergisms (e.g. macro- and micronutrients, C/N ratio, ammonia inhibition, alkalinity) were
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40 unlikely to occur under the trialed experimental conditions. The impact of incoming
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42 microbes (microbes arriving with the substrate) on anaerobic digestion microbial
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44 community and performance is a topic that warrants further research.
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58 Due to BMP tests limitations, continuous experiments are required to better understand the
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60 benefits and constraints of co-digesting microalgae and animal manure. However, few
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4 studies have reported the operation of continuous anaerobic digesters co-treating
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6 microalgae and animal manure (Table 5). Under mesophilic conditions, Wang et al. [67]
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8 co-digested *Chlorella* sp. and pig manure (10/90% VS-basis) at a HRT of 21 days and an
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10 OLR around 1.4 gVS/(L_r·day), Mahdy et al. [66] co-digested *Chlorella vulgaris* and cattle
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12 manure (80/20% VS-basis) at a HRT of 23 days and an OLR of 2.1 gVS/(L_r·day), and
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14 Meneses-Reyes [70] co-digested lipid-spent *Chlorella vulgaris* with glycerol and poultry
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16 litter (30/3/67% dry-basis) at a HRT of 30 days and an OLR of 0.7 gVS/(L_r·day). Despite
17
18 the differences on manure type and OLR, which resulted in quite different pH, TAN, and
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20 NH₃ concentrations, these three studies showed that co-digesting *Chlorella* with manure is
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22 technically feasible under mesophilic conditions. Tsapekos et al. [71] is the only study co-
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24 digesting microalgae (*Nannochloropsis limnetica*) and animal manure under thermophilic
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26 conditions. Tsapekos et al. [71] showed that adding microalgae to a pig manure digester
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28 (40/60% VS-basis) increased the digester methane yield and reduced the concentration of
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30 VFAs. Meneses-Reyes et al. [70] also reported a lower VFA concentration when
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32 microalgae (30/70% dry-basis) or microalgae and glycerol (30/3/67% dry-basis) were co-
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34 digested with poultry litter. The lower VFA concentration under co-digestion conditions
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36 indicates that adding microalgae to an animal manure digester can lead to a more stable
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38 process.
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50 **5. Anaerobic co-digestion of microalgae residues**

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52 The pre-treatment of microalgae has been largely researched since microalgae low
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54 anaerobic biodegradability (extent and rate) is one of the major bottlenecks of microalgae
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56 anaerobic digestion [22, 125, 126]. Microalgae pre-treatment prior to its anaerobic co-
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58 digestion has also been used to improve microalgae biodegradability [20, 49, 55, 66, 90, 91,
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4 123, 127]. However, in many cases, the pre-treatment energy/economic balance is negative,
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6 i.e. the pre-treatment requires more resources than what is recovered from the additional
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8 methane production [22]. Additionally, the impact of the pre-treatment on AD performance
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10 is less evident when microalgae are co-digested than when microalgae are mono-digested
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12 [55, 91]. Consequently, the incentive to pre-treat microalgae prior to its co-digestion is low.
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16 Microalgae pre-treatment (out of the scope of this literature review) have been extensively
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18 reviewed in Jankowska et al. [126] and Passos et al. [22].
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24 A more suitable approach may be to pre-treat microalgae to recover high-value compounds
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26 (e.g. lipids, proteins, antioxidants, pigments) and treat microalgae residues through
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28 anaerobic digestion [23, 128]. Interestingly, several authors have reported that the methane
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30 yield of microalgae residues is between 20 and 100% higher than the methane yield of raw
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32 microalgae [14, 20, 121, 129-131]. However, as highlighted by Astals et al. [50], the
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34 recovery of high-value products will reduce the amount of microalgae diverted to AD and,
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36 consequently, methane yields cannot be used to directly compare the amount of methane
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38 that will be produced in each scenario. Finally, a factor that is not always taken into account
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40 is that microalgae pre-treatment also increases microalgae hydrolysis rate, which further
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42 contributes to improve the methane yield of a continuous AD system. The anaerobic co-
43
44 digestion of microalgae residues after lipid and/or protein extraction with a range of co-
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46 substrates is discussed in the following subsections and summarized in Table 6 and Table 7.
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55 **5.1. Co-digestion of lipid-spent microalgae with glycerol and other co-substrates**

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4 The anaerobic co-digestion of lipid-spent microalgae and glycerol (by-product of biodiesel
5 production) has been investigated by several researchers [14, 16, 51, 52, 81]. The
6
7 integration of biodiesel production from microalgal lipids and the anaerobic co-digestion of
8
9 by-products is a biorefinery approach that aims to make the process more economically
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11 feasible by (i) maximizing the energy recovery from microalgae, (ii) reducing the amount
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13 of residues to be managed, and (iii) reusing the nutrients released during the AD and the
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15 CO₂ from biogas combustion for microalgae cultivation (Fig. 1B).
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25 Ehimen et al. [51], who produced biodiesel from *Chlorella* sp. (oil fraction of 0.27 TS-
26 basis) using both conventional (via solvent extraction) and insitu transesterification,
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28 calculated a maximum yield of 0.028 g of glycerol per g (dry) of *Chlorella* sp. or 0.038 g of
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30 glycerol per g (dry) of lipid-spent *Chlorella* sp. The co-digestion BMP tests carried out
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32 using this relative quantity showed that glycerol addition increased the methane yield by
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34 4% and 7% when co-digested with insitu and conventional lipid-spent microalgae
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36 respectively. These values are in agreement with the values obtained when the experimental
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38 methane yield of the insitu (270 mLCH₄/gTS) and conventional (220 mLCH₄/gTS) lipid-
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40 spent microalgae are combined with the glycerol theoretical methane yield (426
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42 mLCH₄/gTS). Interestingly, the combination of the glycerol maximum yield (0.038 g of
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44 glycerol per g (dry) of lipid-spent *Chlorella* sp.) and a hypothesized volatile-to-total solids
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46 (VS/TS) ratio of 0.8 for the lipid-spent *Chlorella* sp. shows that the addition of glycerol
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48 would only represent a ~5% increase of the digester OLR (VS-basis). In a subsequent
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50 study, Ehimen et al. [16] evaluated the feasibility of co-digesting lipid-spent *Chlorella* sp.
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52 with glycerol in continuous digesters under several treatment conditions (i.e. HRT, OLR,
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4 C/N ratio and temperature). The addition of glycerol to increase the C/N ratio from 5.4
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6 (mono-digestion) up to 12.4 improved the methane yield from 190 to 300 mLCH₄/gVS.
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9 However, when the glycerol dose was further increased to reach a C/N of 24.2, there was a
10
11 reduction of the methane yield linked to the accumulation of VFA. It is worth highlighting
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13 that the amount of glycerol needed to increase the C/N ratio from 5.4 to 12.4 is much
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15 higher than the glycerol generated from microalgal lipids transesterification, with the
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17 literature average being 0.03 g of glycerol per g (dry) of lipid-spent microalgae.
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24 The results obtained by Ramos-Suarez and Carreras [14], who co-digested lipid-spent
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26 *Scenedesmus* sp. with crude glycerol, showed the same trend as Ehimen et al. [16, 51]. On
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28 the one hand, the methane yield of the mixture with the relative proportion between lipid-
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30 spent microalgae and glycerol (0.0235 g of glycerol per g (VS) of lipid-spent *Scenedesmus*
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32 sp.) did not show a significant difference compared to the methane yield of the lipid-spent
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34 microalgae. This is likely due to the small amount of glycerol in the mixture. On the other
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36 hand, larger amounts of glycerol (11% VS-basis) were able to increase the methane yield;
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38 but when the glycerol concentration was further increased (29% VS-basis) the test showed
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40 clear signs of inhibition. From Ehimen et al. [16] and Ramos-Suarez and Carreras [14]
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42 results, it can be concluded that a lipid-spent microalgae digester is capable of accepting all
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44 crude glycerol produced during the biodiesel production and still has capacity to accept
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46 other suitable co-substrates. This organic and volumetric loading spare capacity could be
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48 used to digest other waste and further improve the biorefinery economic feasibility.
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58 Besides glycerol, lipid-spent microalgae have been co-digested with other wastes, such as
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60 FOG [120], waste activated sludge [81], food waste leachate [132], pig manure [50],
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4 poultry litter [70, 122], and cellulose [131]. Most of these studies have been carried out
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6 using BMP tests, however, results suggest that the co-digestion of lipid-spent microalgae
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8 with other co-substrates, particularly carbon-rich wastes, is not antagonistic. Therefore, the
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10 co-substrate loading rate and subsequent methane production improvement will depend on
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12 the (i) AD plant capacity, (ii) co-substrate availability and biodegradability, (iii) secondary
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14 inhibitors, and (iv) the impact of the co-substrates on supernatant and digestate quality.
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16 However, as previously discussed, most of these factors can only be reliably evaluated in
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18 continuous experiments. Park and Li [120], who operated a continuous digester co-
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20 digesting lipid-spent *Nannochloropsis salina* and FOG, observed that the addition of FOG
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22 allowed to increase the OLR from 2 to 3 gVS/(L_r·day) whereas the control reactor
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24 (microalgae residues only) was inhibited when the same OLR change occurred; likely due
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26 to ammonia inhibition. The co-digester was inhibited when the OLR was subsequently
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28 increased to 4 gVS/(L_r·day); likely due to LCFA inhibition. Park and Li [120] results
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30 showed a clear synergy between *Nannochloropsis salina* and FOG since microalgae
31
32 provided alkalinity and nutrients while FOG boosted the methane production and diluted
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34 ammonia concentration. However, Park and Li [120] results also showed that there was a
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36 risk associated with the addition of a co-substrate, particularly when a certain threshold is
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38 surpassed. The benefits and constraints of using FOG as co-substrate have already been
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40 discussed by Long et al. [115] and Mata-Alvarez et al. [47].
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53 **5.2. Co-digestion of protein-spent microalgae**

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55 The anaerobic co-digestion of protein-spent microalgae has received less attention than the
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57 co-digestion of lipid-spent microalgae. This is likely due to (i) the past few years' interest
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59 on the production of microalgal-derived biodiesel [11, 17], and (ii) the lower production
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4 costs and higher nutritional value obtained when the whole microalgal biomass is used as
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6 feed source [8, 133]. However, protein hydrolyzates have several applications in the food
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8 and drink industry (e.g. sport drinks) and the fermentation industry [14, 133]. Additionally,
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10 the extraction of proteins would reduce the risk of ammonia inhibition associated with
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12 microalgae anaerobic digestion.
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18 To the best of our knowledge, only Ramos-Suarez and Carreras [14] and Astals et al. [50]
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20 have studied the anaerobic co-digestion of protein-spent microalgae. Ramos-Suarez and
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22 Carreras [14] co-digested protein-spent microalgae with paper sludge and *Opuntia maxima*,
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24 while Astals et al. [50] co-digested protein-spent microalgae with pig manure. Although
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26 both studies used *Scenedesmus* sp., the method used to release the protein was different
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28 since Ramos-Suarez and Carreras [14] used an enzymatic pre-treatment and Astals et al.
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30 [50] used free nitrous acid (chemical pre-treatment). Both studies observed that the
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32 extraction of protein significantly increased microalgae's methane yield from 140 to 273
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34 mLCH₄/gVS [14] and from 163 to 222 mLCH₄/gVS [50]. However, Astals et al. [50] also
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36 showed that protein extraction reduced by 54% the amount microalgae diverted to
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38 anaerobic digestion, while lipid extraction only reduced it by 14%. The amount of proteins
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40 and lipids in microalgae varies depending on the microalgae strain and cultivation
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42 conditions, however, microalgae are typically characterized by a larger proportion of
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44 proteins than lipids [18, 133]. Consequently, the need to implement anaerobic co-digestion
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46 is even more important when protein-spent microalgae is treated by anaerobic digestion.
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58 **6. Conclusions**

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4 Anaerobic co-digestion is an opportunity to overcome the drawbacks of microalgae mono-
5 digestion and boost the methane production of microalgae and microalgae residues biogas
6 plants. Microalgae can also be used as co-substrate that, besides increasing the digester
7 organic loading rate, can represent a valuable source of alkalinity, macro- and
8 micronutrients. Microalgae have been co-digested with a large variety of co-substrates,
9 such as sewage sludge, animal manure, food waste, crops, glycerol, paper waste, and fat, oil
10 and grease. Most studies have focused on the impact of co-digestion on the methane yield,
11 while less attention has been paid to other important factors, such as supernatant nutrient
12 content, digestate dewaterability, biosolids quality and H₂S concentration in the biogas.
13 Sewage sludge is the most researched co-substrate since the cultivation of microalgae in
14 wastewater treatment plants could be used as secondary, tertiary and anaerobic digestion
15 supernatant treatment. For microalgae biogas plants, highly biodegradable carbon-rich
16 wastes are the preferred co-substrates since they can increase the digester organic loading
17 rate and methane yield without increasing the nitrogen concentration. Several studies
18 optimized the co-substrate dose by balancing the C/N ratio, however, positive interactions
19 occurring during anaerobic co-digestion could not always be linked to the C/N ratio
20 indicating that other factors have to be considered. Overall, more lab and pilotscale
21 continuous experiments are needed to get a holistic understanding of microalgal anaerobic
22 co-digestion.
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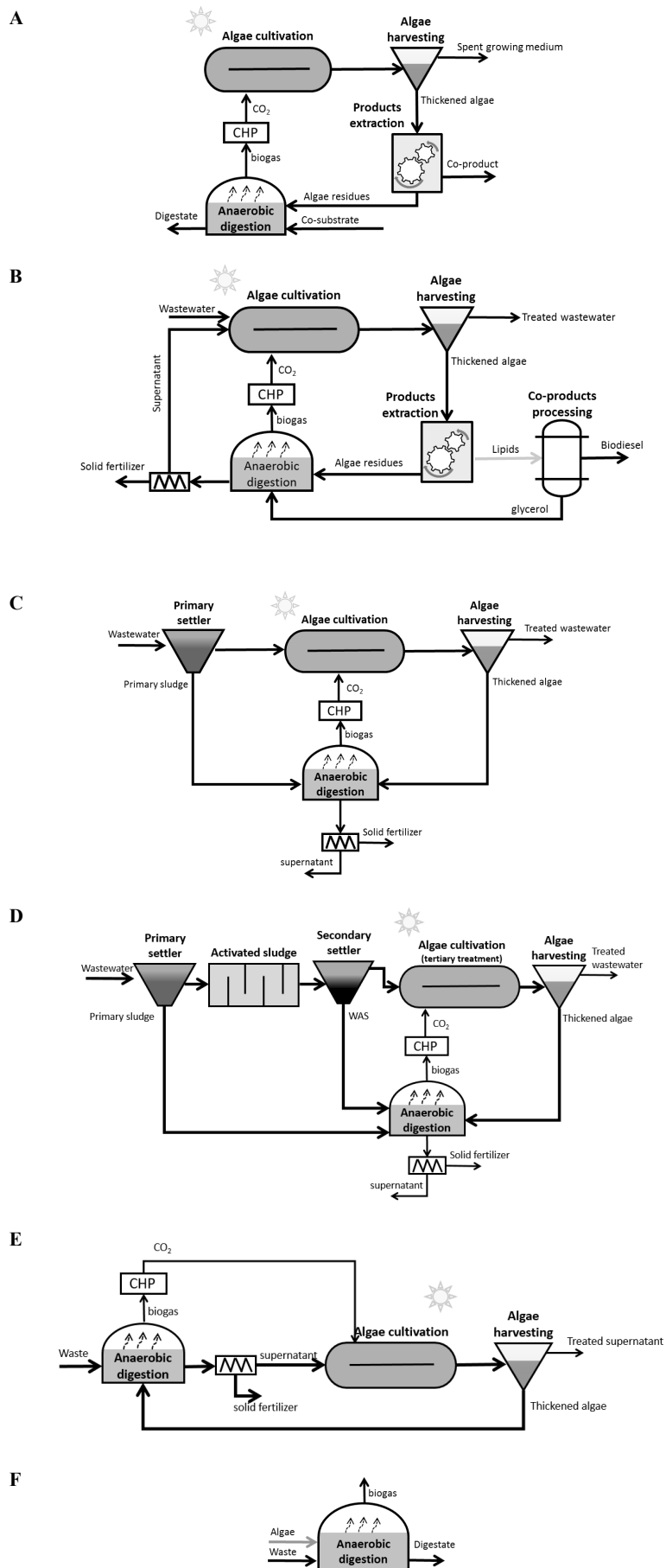


Fig. 1. Most researched scenarios for microalgae anaerobic co-digestion: (A) high-value products biorefinery, (B) biodiesel biorefinery, (C) HRAP as secondary treatment in a wastewater treatment plant, (D) photobioreactor as tertiary treatment in a wastewater treatment plant, (E) HRAP as anaerobic digestion supernatant treatment, and (F) microalgae as co-substrate in an existing biogas plant.

Table 1. Summary of sewage sludge and microalgae co-digestion studies in BMP tests

Microalgae	Co-substrate	Mixture ratio	T (°C)	Methane yield with co-digestion (mLCH ₄ /gVS)	Improvement ^a (%)	Reference
<i>Chlorella</i> sp.	WAS	41:59 (TS)	37	468 ^b	23	Wang et al. [85]
<i>Chlorella</i> sp. & <i>Scenedesmus</i> sp.	Sewage sludge	37:63 (TS)	37	408	n.d.	Olsson et al. [62]
<i>Isochrysis galbana</i>	Sewage sludge	12:88 (TS)	55	378	n.d.	
<i>Selenastrum capricornutum</i>	Sewage sludge	25:75 (VS)	33	413 ^b	- 8	Caporgno et al. [82]
<i>Chlorella vulgaris</i>	WAS	50:50 (VS)	33	392 ^b	9	Caporgno et al. [82]
<i>Chlorella vulgaris</i> (pretreated)	Primary sludge	75:25 (COD)	35	107 ^c	6	Mahdy et al. [20]
Lipid-spent <i>Botryococcus braunii</i>	WAS	50:50 (COD)	35	283 ^c	16	Mahdy et al. [20]
<i>Micractinium</i> sp.	WAS	75:25 (COD)	35	293 ^c	13	
<i>Chlorella</i> sp.	WAS	75:25 (VS)	35	393	7	Neumann et al. [81]
<i>Chlorella sorokiniana</i>	WAS	21:79 (VS)	37	236	0	Wang and Park [6]
<i>Scenedesmus quadricauda</i>	WAS	21:79 (VS)	37	253	5	
Microalgal biomass ^d	WAS	25:75 (VS)	37	442	26	Beltran et al. [79]
<i>Chlorella</i> sp.	Primary sludge	49:51 (VS)	35	222	12	Garoma and Nguyen [63]
<i>Chlorella sorokiniana</i> & <i>Scenedesmus</i> sp.	Septic sludge	92.5:7.5 (VS)	35	168 ^b	n.d.	Hlavinek et al. [54]
	WAS ^e	50:50 (VS)	35	547	84	Lu and Zhang [84]
	WAS ^f	9:91 (VS)	37	560	28	Wagner et al. [86]
	WAS (pretreated)	9:91 (VS)	37	400	11	
<i>Scenedesmus</i> sp. (pretreated)	WAS (pretreated)	20:80 (VS)	37	187	- 2	Arias et al. [60]
<i>Spirulina platensis</i>	Sewage sludge	67:37 (VS)	35	343	15	Du et al. [83]
<i>Scenedesmus</i> sp. & <i>Chlorella</i> sp.	Sewage sludge	40:60 (VS)	35	237	2	Olsson et al. [34]
Microalgal biomass ^d	Primary sludge	25:75 (VS)	37	291	- 5	Solé-Bundó et al. [55]
Microalgal biomass ^d (pretreated)	Primary sludge	25:75 (VS)	37	339	6	

^a The methane yield improvement is calculated as the product summation of each substrate methane yield, considering the co-substrates proportion and their experimental methane yield in mono-digestion; ^b Expressed as mLbiogas/gVS; ^c Expressed as mLCH₄/gCOD; ^d Microalgae collected from a WWTP; ^e Obtained from the anaerobic phase of an enhanced biological phosphorous removal system; ^f Obtained from the aerobic phase of an enhanced biological phosphorous removal system; n.d. stands for not defined

Table 2. Summary of sewage sludge and microalgae co-digestion in continuous lab-scale reactors

Microalgae	Co-substrate	Mixture ratio	AD operation	T (°C)	Working volume (L)	OLR (gVS/(L _r ·day))	HRT (days)	Methane yield (LCH ₄ /gVS)	Reference
<i>Spirulina maxima</i>	Sewage sludge	50:50 (VS)	Continuous	35	1.5	3.9	20	0.36	Samson and LeDuy [78]
<i>Scenedesmus</i> sp.	Sewage sludge	25:75 (VS)	Semi-continuous ^a	35	0.45	0.5 ^b	15	0.39 ^c	Peng and Colosi [12]
Microalgal biomass ^d	Sewage sludge	50:50 (VS)	Semi-continuous ^a	35	0.45	0.5 ^b	15	0.51 ^c	Peng and Colosi [12]
<i>Chlorella</i> sp.	Sewage sludge	50:50 (VS)	Continuous	55	10	n.d.	28	0.44 ^c	Hidaka et al. [64]
<i>Scenedesmus</i> sp. & <i>Chlorella</i> sp.	Sewage sludge	4-15:96-85 (v/v)	Semi-continuous	37	5	2.4	15	0.20	Olsson et al. [34]
Microalgal biomass ^d	Primary sludge	25:75 (VS)	Semi-continuous	35	1.5	3.5	10	0.17	Solé-Bundó et al. [55]

^a Feed every 48 hours; ^b Expressed as gVSS/(L_r·day); ^c Expressed as LCH₄/kgVSS; ^d Microalgae collected from a WWTP; n.d. stands for not defined

Table 3. Summary of agri-industrial wastes and microalgae co-digestion studies in BMP tests

Microalgae	Co-substrate	Mixture ratio	T (°C)	Methane yield (mLCH ₄ /gVS)	Improvement ^a (%)	Reference
Taihu blue algae	Corn straw	n.d.	35	325	62	Zhong et al. [72]
<i>Spirulina platensis</i>	Switchgrass	33:67 (VS)	35	198	-2	El-Mashad [68]
<i>Nannochloropsis salina</i>	Corn silage	33:67 (VS)	50	236	3	
	Corn cob	14:86 (v/v)	37	660 ^c	15	Schwede et al. [49]
		25:75 (v/v)		610 ^c	18	
<i>Dunaliella salina</i>	Olive mill solid waste	50:50 (VS)	35	285	48	Fernández-Rodríguez et al. [111]
<i>Scenedesmus</i> sp.	<i>Opuntia maxima</i>	25:75 (VS)	37	234	65	Ramos-Suárez et al. [110]
<i>Scenedesmus</i> sp.	<i>Opuntia maxima</i>	10:90 (VS)	37	166	7	
<i>Spirulina platensis</i>	Pretreated switchgrass	50:50 (TS)	50	354	10	El-Mashad [117]
<i>Botryococcus braunii</i>	Glycerol	90:10 (VS)	37	430	9	Neumann et al. [81]
<i>Chlorella</i> sp. & <i>Scenedesmus</i> sp.	<i>Sida hermaphrodita</i>	40:60 (VS)	35	352	56	Dębowski et al. [32]
<i>Chlorella</i> sp. & <i>Monoraphidium</i> sp.	Wheat straw	50:50 (VS)	37	289	6	Solé-Bundó et al. [91]
<i>Scenedesmus acuminatus</i> & <i>Scenedesmus quadricauda</i>	Cheese whey (deproteinated)	17:83 (VS)	35	302	-9	Carminati et al. [35]
	Cellulose	16:84 (VS)		272	-5	
<i>Chlorella</i> sp. ^d	Coffee husks ^d	14:86 (VS)	35	196	87	Passos et al. [90]

^a The methane yield improvement is calculated as the product summation of each substrate methane yield, considering the co-substrates proportion and their experimental methane yield in mono-digestion; ^b Expressed as mLCH₄/gTS; ^c Expressed as mLbiogas/gVS; ^d pretreated at 120 °C; n.d. stands for not defined

Table 4. Summary of agri-industrial wastes and microalgae co-digestion in mesophilic continuous lab-scale reactors

Microalgae	Co-substrate	Mixture ratio	OLR (gVS/(L _r ·day))	HRT (days)	Methane yield (LCH ₄ /gVS)	Reference
<i>Nannochloropsis salina</i>	Corn silage	14:86 (v/v)	2	n.d.	1.0-1.5 ^a	Schwede et al. [49]
<i>Nannochloropsis salina</i> (pretreated)	Corn silage	14:86 (v/v)	2	n.d.	1.5-1.8 ^a	Schwede et al. [49]
<i>Scenedesmus</i> sp.	<i>Opuntia maxima</i>	25:75 (VS)	2	30	0.21	Ramos-Suárez et al. [110]
			4	15	0.29	
			6	10	0.20	
<i>Scenedesmus</i> sp.	<i>Opuntia maxima</i>	25:75 (VS)	2	40	0.32	Ramos-Suárez et al. [110]
			4	20	0.30	
			5	15	0.31	
			7	12	0.28	
<i>Chlorella</i> sp. & <i>Monoraphidium</i> sp.	Wheat straw	50:50 (VS)	1	20	0.21	Solé-Bundó et al. [91]
<i>Monoraphidium</i> sp. & <i>Chlorella</i> sp. (pretreated)	Wheat straw (pretreated)	50:50 (VS)	1	20	0.24	Solé-Bundó et al. [91]
<i>Stigeoclonium</i> sp. and <i>Scenedesmus</i> sp.	Papaya waste	50:50 (w/w)	1	31	0.55	Cea-Barcia et al. [118]
<i>Scenedesmus acuminatus</i> & <i>Scenedesmus quadricauda</i>	Cheese whey (deproteinated)	17:83 (VS)	1.5 ^b	30	0.22	Carminati et al. [35]
	Cellulose	16:84 (VS)	1.5 ^b	30	0.12	

^a Expressed as Lbiogas/(L_r·day); ^b Expressed as gCOD/(L_r·day); n.d. stands for not defined

Table 5. Summary of animal manure and microalgae co-digestion studies in batch and continuous conditions

Microalgae	Co-substrate	AD operation	Mixture ratio	T (°C)	Methane yield (mLCH ₄ /gVS)	Improvement ^a (%)	Reference
<i>Chlorella vulgaris</i> & <i>Scenedesmus obliquus</i>	Pig manure	BMP	50:50 (COD)	35	220	15	Gonzalez-Fernandez et al. [65]
<i>Chroococcus</i> sp.	Cattle manure	BMP	50:50 (VS)	36	292	70	Prajapati et al. [121]
<i>Scenedesmus</i> sp.	Pig manure	BMP	15:85 (VS)	37	300	0	Astals et al. [50]
			30:70 (VS)	37	296	9	
			50:50 (VS)	37	260	11	
<i>Chlorella</i> sp.	Pig manure	BMP	6:94 (VS)	35	348	11	Wang et al. [67]
		Continuous	10:90 (VS)	35	190	0 ^b	
<i>Chlorella</i> 1067	Chicken manure	BMP	20:80 (VS)	35	239	31	Li et al. [9]
<i>Chlorella vulgaris</i> (pretreated)	Cattle manure	BMP	80:20 (VS)	55	431	10	Mahdy et al. [66]
		Continuous	80:20 (VS)	37	351	n.d.	
<i>Oscillatoria tenuis</i>	Pig manure	BMP	33:67 (VS)	35	191	n.d.	Cheng et al. [123]
<i>Scenedesmus</i> sp. & <i>Chlorella</i> sp.	Pig manure	BMP	50:50 (v:v)	25-31	326	n.d.	Panyaping et al. [124]
<i>Scenedesmus</i> sp. & <i>Chlorella</i> sp. (pretreated)	Pig manure (pretreated)				292	n.d.	
<i>Nannochloropsis limnetica</i>	Pig manure	BMP	40:60 (VS)	53	355	12	Tsapekos et al. [71]
		Continuous			216	n.d.	

^a The methane yield improvement is calculated as the product summation of each substrate methane yield, considering the co-substrates proportion and their experimental methane yield in mono-digestion; ^b No significant differences were observed when compared to pig manure mono-digestion; ^c Result compared to mono-digestion of chicken litter; n.d. stands for not defined. Including reference [123] and [124].

Table 6. Summary of microalgae residues co-digestion studies in BMP tests

Microalgae	Co-substrate	Mixture ratio	T (°C)	Methane yield (mLCH ₄ /gVS)	Improvement ^a (%)	Reference
Lipid-spend microalgae:						
<i>Chlorella</i> sp.	Glycerol	67:3 (v/v)	37	267 ^b	4-7	Ehimen et al. [51]
<i>Scenedesmus</i> sp.	Glycerol	89:11 (VS)	37	255	n.d.	Ramos-Suárez and Carreras [14]
<i>Nannochloropsis gaditana</i>	Cellulose	50:50 (VS)	37	268	n.d.	Barontini et al. [131]
<i>Chlorella vulgaris</i>	Chicken litter & glycerol	30:67:3 (TS)	37	131	16 ^c	Meneses-Reyes et al. [122]
Protein-spend microalgae:						
<i>Scenedesmus</i> sp.	Paper sludge	74:26 (VS)	37	173	35	Ramos-Suárez and Carreras [14]
<i>Scenedesmus</i> sp.	Pig manure	15:85 (VS)	37	319	5	Astals et al. [50]
		30:70 (VS)	37	302	4	

^aThe methane yield improvement is calculated as the product summation of each substrate methane yield, considering the co-substrates proportion and their experimental methane yield in mono-digestion; ^b Expressed as mLCH₄/gTS; ^c Result compared to mono-digestion of chicken litter; n.d. stands for not defined

Table 7. Summary of microalgae residues co-digestion studies in mesophilic continuous lab-scale reactors

Microalgae	Co-substrate	Mixture ratio	OLR (gVS/(L _r ·day))	HRT (days)	Methane yield (LCH ₄ /gVS)	Reference
Lipid-spent <i>Nannochloropsis salina</i>	FOG	33:67 (VS)	2	40	0.45	Park and Li [120]
			4	20	0.12	
			6	13	fail	
Lipid-spent <i>Nannochloropsis salina</i>	FOG	50:50 (VS)	2	40	0.40	Park and Li [120]
			4	20	0.54	
Lipid-spent <i>Nannochloropsis salina</i>	FOG	67:33 (VS)	2	40	0.38	Park and Li [120]
			4	20	0.28	
Lipid-spent <i>Chlorella vulgaris</i>	Chicken litter & glycerol	30:67:3 (VS)	0.7	30	0.27	Meneses-Reyes et al. [70]