Chapter 4

# The Influence of Microalgae Addition as Co-Substrate in Anaerobic Digestion Processes

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**Abstract**

Growth microalgae could be used as co-substrates in anaerobic digestion processes to produce biogas of a high-calorific value, which could be expended as heat or electricity in cogeneration engines. Lignocellulosic and high-carbon content wastes, due to their characteristics, hinder anaerobic digestion processes. The use of microalgae as a co-substrate with high-carbon content residues can adjust the C/N ratio and thereby obtain, in some cases, a higher biogas production and greater biodegradability of wastes during anaerobic digestion than without co-digestion options. In addition, microalgae and cyanobacteria are photosynthetic microorganisms that can produce oxygen and oxidize the organic matter and $NH_4^+$ contained in wastewaters. The growth of microalgae in industrial effluents and wastewaters can considerably reduce the organic matter contained in them and their pollutant load. This growth can take advantage of the nutrients that still remain in industrial effluents, avoiding the use of clean water for the growth of biomass. The chapter will focus on an overview of microalgae anaerobic co-digestion with different wastes and the benefits of this option.

**Keywords:** microalgae, wastewaters, biogas, anaerobic digestion, microalgae growth

## 1. Introduction

One of the main challenges that society will face in the near future is the potential lack of traditional energy sources. The rising price of fossil-based fuels and their negative environmental impact combined with increasing energy consumption make the demand for renewable energy sources greater. For this reason, a wide variety of biomass has been investigated

in order to evaluate its potential as a proper feedstock for the production of different biofuels, such as biodiesel, bio-methanol, bio-hydrogen, bio-oil, and biogas [1]. Nevertheless, the increasing world population will need an adequate food supply, which could be a problem if cultivated land is destined to biofuels and not to human or animal feed. Thus, non-edible biomass, which does not require usable land, would be a promising alternative. In this regard, the attention of the scientific community has been focused on oleaginous microorganisms, such as microalgae and cyanobacteria, in recent years. Microalgae do not need agricultural land for growing, and they improve air quality through $CO_2$ removal and require minimal use of fresh water resources [2].

The main properties that make some microalgae and cyanobacteria good alternatives as biomass for biofuel production include their highly efficient photosynthetic mechanisms [3]; their elevated biomass production of up to 5–10% vs. 0.5–3% in plants [4]; their growth rates, which are 5–10 times faster than land-based feedstock [5]; and their accumulation of lipids and carbohydrates [1, 6]. However, the main nutrients required for the growth of microalgae and cyanobacteria are inorganic carbon (some microalgae species are able to utilize organic carbon), inorganic nitrogen (ammonium or nitrate), and phosphorous. These requirements can make their growth expensive in some cases. For example, generating 1 kg of biodiesel in fresh water requires 3,726 kg of water, 0.33 kg of nitrogen, and 0.71 kg of phosphate [7]. However, it is now known that microalgae can be grown using nutrient-rich wastewaters like digestates from anaerobic digestion processes such as liquid supernatants rich in nitrogen and phosphorous, animal manure or textile wastewater [8], food wastewater [9], and aquaculture wastewater [10], among others. Even in saline waters, which are usually rich in nitrogen [11], this disadvantage to the water quality for growth is easily overcome. In the same way, recycling harvest water reduces the water and nutrient requirements by 84 and 55%, respectively [7]. The use of wastewater for microalgae and cyanobacteria growth presents the advantage of reducing the cost and environmental impact of the system by reducing the use of clean water and mineral nutrients while biomass productivity is comparable to that obtained from a synthetic medium [12].

Microalgae also uptake carbon by the photosynthetic process during growth, reducing $CO_2$ emissions 10 times more efficiently than those reduced in a forest [6, 13], by transforming $CO_2$ into new biomass. Microalgae culture can contribute simultaneously to both $CO_2$ fixation and wastewater treatment [14]. Hirata et al. [15] found that a batch culture of *Chlorella* sp. UK001, using sunlight as a light source and growing at a mesophilic temperature with pH between 5.5 and 6.0, achieved a mean rate of $CO_2$ fixation during the culture of 0.0318 g $CO_2$/L·d. Maeda et al. [16] found that another strain of *Chlorella*, strain *Chlorella* sp. T-1, was an ideal candidate for the biological fixation of $CO_2$ exhausted by a coal-fired thermal power plant. Other authors demonstrated that the strain *Chlorella* sp. MTF-15 could efficiently utilize $CO_2$, $NO_x$, and $SO_2$ from the different flue gases obtained in a steel plant: flue gas from a coke oven, flue gas from a hot stove, and flue gas from a power plant for cultivation [17].

Furthermore, the growth of microalgae in wastewaters aids in the treatment of pollutant wastewaters and could be introduced as a tertiary treatment [18–22]. In addition, the capacity of microalgae for synthesizing and accumulating different compounds, which could be

considered for pharmaceutical and nutraceutical purposes, is an added value [23]. The different metabolic pathways of fresh and marine water algae provide promising sources of fatty acids, steroids, carotenoids, polysaccharides, lectins, and halogenated compounds, among others [24]. Microalgae are the most promising sources of pigments and natural carotenoids of commercial interest, including β-carotene, lutein, and astaxanthin [25, 26]. Furthermore, the carotenoids produced by microalgae are devoid of the toxic effects associated with synthetic derivatives [26]. Microalgae are also used as nutritional supplements for animals and humans because of the quality of proteins that they produce. *Spirulina*, *Chlorella*, *Dunaliella*, and *Nostoc* are microalgae and cyanobacteria grown for human consumption [25].

The most common systems for the cultivation of microalgae used for biogas production are open pond reactors (OPRs), photobioreactors (PBRs), and hybrid systems. OPRs are relatively low-cost systems, although the biomass yield is lower and contamination is quite common. PBR systems permit a higher control over microalgae growth and its optimization; nevertheless, the cost of these systems is much higher than OPRs [6].

Different approaches to microalgae as biomass for biofuel extraction have been studied, but not all of them with the same success. Regarding lipid accumulation for biodiesel production, algae grown in wastewater typically showed lipid mass fractions in the volatile suspended solids (VSSs) in the range of 4.9–11.3%. This fraction is much lower than that recommended for economical biodiesel production [27]. In order to enhance the energy potential of microalgae and cyanobacteria, anaerobic digestion has been studied [1, 6] as another alternative. Anaerobic digestion is a complex biological process in which organic raw materials are converted to biogas through the action of a consortium of microorganisms that are sensitive to or completely inhibited by oxygen. Biogas is a mixture of methane (60–70%) and carbon dioxide (30–40%) and traces of other constituents (hydrogen, hydrogen sulfide, etc.) of high-energetic value from 20 to 25 MJ/$m^3$ [28]. Around 31 $m^3$ of methane per 100 kg of chemical oxygen demand (COD) fed into an anaerobic reactor can be produced, with a maximum energetic value of 108 kWh as electric energy or 308 kWh as heat. It has been reported in the literature that microalgae and cyanobacteria can be potentially used for energy recovery through anaerobic digestion, although the yields obtained depend highly on the species and the operational conditions of growth [1, 29]. The initial studies in the 1950s [30] obtained values of methane yields of 0.17–0.32 L $CH_4$/g $SV_{added}$ for *Chlorella* and *Scenedesmus* in batch processes, although other authors found higher values of methane yield at 0.587 L $CH_4$/g $SV_{added}$ and 0.505 L $CH_4$/g $SV_{added}$ for *Chlamydomonas reinhardtii* and *Dunaliella salina*, respectively [29].

Growth conditions could affect the morphology and intracellular substances in microalgae. The thickness of the cell walls in microalgae could be increased due to stressed growing conditions, which could be a disadvantage during anaerobic digestion [27]. In addition, microalgae and cyanobacteria present a low C/N ratio, which could lead to an ammonium accumulation and result in an inhibition of the digestion process. Samson and LeDuy [31] reported concentrations of ammonia of up to 7000 mg/L for the anaerobic digestion of the protein-rich cyanobacteria *Spirulina maxima*. However, the use of microalgae as co-substrate with other substrates or feedstocks in anaerobic co-digestion processes can improve these limitations and bring certain benefits. Anaerobic co-digestion is the simultaneous anaerobic

digestion of two or more substrates, and it is a proven approach to overcome the drawbacks of single digestion [32]. Mata-Alvarez et al. [33] in the year 2000 already wrote: "The use of a co-substrate, in most cases improves biogas yield due to positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates." Co-digestion has several advantages as follows: adjusting the C/N ratio, improving the nutrients, and diluting the inhibitor compounds [34]. The co-digestion of microalgae with high-carbon biomass leads to a better balanced substrate for anaerobic digestion [12, 13, 27]. Nevertheless, there are some problems that must be solved, such as the breakage of the thick cellular walls in some microalgae and cyanobacteria. Prospective methods could be different kinds of pretreatments before anaerobic digestion in some particular cases.

Nonetheless, due to the high variety of microalgae and cyanobacteria and the wide range of different uses, it is not clear yet what the most effective process for biofuel production is. Although to this respect, some authors suggest that the direct use of microalgae or cyanobacteria in an anaerobic co-digestion process is the best choice, while other researchers propose that the best choice is to produce biofuel as a first step followed by an anaerobic digestion of the residual by-products [1]. This chapter aims at providing a current perspective of microalgae exploitation as biomass in anaerobic digestion and co-digestion processes and shows the advantages of their growth in wastewater and anaerobic digestates.

## 2. Microalgae growth in wastewaters

The cultivation of microalgae in wastewater has long been recognized as a viable option for sustainable biomass production and wastewater treatment [18–21]. The main nutritional requirements for microalgae growth include nitrogen, phosphorus, and micronutrients such as iron, magnesium, and calcium, which are present in wastewater. Recent developments in microalgal research have demonstrated that microalgae have the required metabolic potential to effectively reduce high concentrations of nutrients such as carbon, phosphorous, and nitrogen present in different wastewater streams [21]. Some species of microalgae have the ability to take up other pollutants, such as heavy metals and harmful chemicals [20]. Therefore, microalgae can be used to serve a dual purpose for the treatment of wastewater as well as generating biomass for various applications because microalgae are rich in carbohydrates, proteins, and lipids.

Various wastewater streams including municipal, industrial, agricultural wastewater, as well as primary and secondary effluent, centrate, and anaerobic digestion effluent were exploited as suitable nutrient media for microalgae cultivation. Each wastewater stream has its own characteristics and challenges such as nutrient variability and the presence of potential inhibitors that could impact microalgal growth. Recently, many investigations have been developed to overcome challenges such as low nutrients, high turbidity, bacterial contamination, and specific toxic materials associated with different wastewaters.

The types of wastewater utilized for algae cultivation also affect the scope of biomass for various applications [21].

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An alternative for recovering energy from microalgae is based on the application of anaerobic digestion processes [35]. In such processes, all organic matters (proteins, carbohydrates, and lipids) present in microalgae biomass would be converted into methane and carbon dioxide (biogas). Several advantages are recognized when energy production from whole microalgae through biogas generation is considered: biogas production involves high-energy yields; biogas production would not require microalgae biomass drying (it involves wet fermentation); biogas can be used to produce heat and electricity through co-generation; microalgae cultures can be used for biogas upgrading (i.e. $CO_2$ biosequestration); and so on. However, some microalgae have a very low C/N ratio, which hinders and inhibits a further anaerobic digestion. Ammonia toxicity and recalcitrant cell walls are commonly cited causes of the low-methane yields found in the anaerobic digestion of some microalgae [36]. Moreover, anaerobic co-digestion of microalgae with other types of biomass such as solid and liquid wastes is quite feasible [35]. The benefits of co-digestion lie in balancing the C/N ratio in the co-substrate mixture, as well as macro and micronutrients, pH, inhibitor/toxic compounds, and dry matter [37].

The main phyla (and species) of microalgae that are being used for biogas production through anaerobic digestion and co-digestion processes are as follows [20, 21, 38]:

- **Chlorophytes**, such as *Chlorella* sp./*vulgaris/sorokiniana*; *Scenedesmus* sp./*quadricauda/obliquus*; *D. salina*; *Nannochloropsis salina*; *Botryococcus braunii*; *Micractinium* sp.; and *Selenastrum capricornutum*.
- **Haptophytes,** such as *Isochrysis galbana*.
- **Cyanobacteria,** such as *Arthrospira platensis* and *Oscillatoria tenuis*.
- **Binary and mixed culture systems:** In mixed culture systems, different microorganisms develop a synergetic relationship and live together by benefiting each other. For instance, in a binary system, a photosynthetic microalga is grown with a heterotrophic microalga or bacteria. In this matrix, microalgae produce oxygen and organic compounds, which are utilized by co-existing heterotrophic microorganisms.

## 2.1. Chlorophytes

### *2.1.1. Chlorella genus*

The growth of the green algae *Chlorella* sp. in wastewater after primary settling of a local municipal wastewater treatment plant was evaluated by Wang et al. [39]. They observed a growth rate of 0.429 $d^{-1}$ with excellent removal of ammonium ($NH_4^+$-N) (74.7%), P (90.6%), and COD (56.5%). These authors also investigated the growth of *Chlorella* sp. using different phases (raw, secondary, and centrate) and demonstrated that the growth rate of microalgae and nutrient removal efficiencies was proportional to the nutrient concentration of the wastewater selected for its cultivation with the highest growth in centrate followed by raw wastewater. Osundeko and Pittman [40] reported a high-sodium concentration of 400 mg/L in sludge liquor/centrate, which can be toxic to freshwater microalgal species, though some

*Chlorella* sp. are tolerant to salinity. More recently, Lu et al. [41] evaluated the biomass productivity and nutrient removal capacity of *Chlorella* sp. in raw dairy wastewater using both indoor bench-scale and outdoor pilot-scale photobioreactors. Results from this study have shown a higher biomass productivity of 260 mg/(L·d) and high nutrient (N and P) removal (83.3 and 38.3 mg/(L·d), respectively) in indoor bench-scale cultures when compared to outdoor pilot-scale cultures with biomass of 110 mg/(L·d) and nutrient removal of 41.3 mg/(L·d) for N and 6.5 mg/(L·d) for P. These differences could have resulted due to the uncontrolled environmental and operational factors that might have affected the microalgae growth during outdoor cultivation.

Nutrient limitation is one of the key challenges for microalgal cultivation in secondary/tertiary wastewater. The supplementation of nutrients is proposed as an alternative method to overcome the nutrient limitations in wastewater. In this sense, Cabanelas et al. [42] identified the potential of coupling a wastewater treatment plant effluent with glycerol for supporting the mixotrophic production of *Chlorella vulgaris* and *Belippo terribilis*. The cultivation of *C. vulgaris* in mixotrophic mode was also studied in a mixture of primary and secondary wastewaters with different ratios (25, 50, and 75 vol.% of the primary wastewater). It was observed that using 25% of the primary wastewater and 75% of secondary wastewater resulted in 100% of COD removal, 100% of ammonium removal, and 82% of nitrate removal [43].

Recently, Ansari et al. [44] studied the cultivation of *Chlorella sorokiniana* in aquaculture wastewater with sodium nitrate supplementation and observed comparable biomass yields to the synthetic medium. In their study, they also observed high ammonia, nitrate, COD, and phosphate removal and proposed that treated water can be redirected toward aquaculture. The biomass obtained in this study showed sufficient lipid, carbohydrate, and protein concentrations to be used as feed supplement. Ramanna et al. [45] supplemented 1.5 g/L urea as a cheap N source for the cultivation of *C. sorokiniana* and achieved a biomass production of 0.218 g/L. A supplementation strategy can yield high-biomass productivities; however, it depends on the nutrient composition of the wastewater used and the requirements of the selected microalgal strain.

For the realization of microalgal $CO_2$ capture and utilization, the selection of microalgal species tolerant to $CO_2$ from various environments and the characterization of growth influencing environmental factors are required [46]. The proper selection of species and optimized cultivation conditions, i.e., light intensity, temperature, nutrient availability, and pH, can maximize $CO_2$ sequestration. *Chlorella* sp. has been widely reported to possess good carbon sequestration potential. Previous studies have obtained hydrocarbons from microbial lipids for their conversion into sustainable fuels as a substitute for fossil hydrocarbons. Furthermore, microalgae have significant applications in the production of valuable materials in the food and pharmaceutical industries, resulting in a high value-added process in the biosequestration of $CO_2$ [46].

Microalgae with a lipid content of lower than 40% of their dry weight make the anaerobic digestion route more feasible than biodiesel in terms of energy recovery. Ras et al. [47] proposed coupling the process of microalgal biomass production and anaerobic digestion. In this process, *C. vulgaris* was cultivated using the nutrient-rich digestate from an anaerobic

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digester; the microalgal biomass was then anaerobically digested to produce methane. In a later study, with hydraulic retention time (HRT) of 28 days, 51% COD removal and methane production of 240 mL/g VSS were achieved. The use of microalgae as a feedstock for bioethanol production is considered to be a sustainable approach to bioethanol production. Microalgal species such as *Chlorella* store energy in the form of starch [48]. The starch accumulated in the microalgae can be easily hydrolyzed to glucose using chemical or enzymatic method. The sugar produced can be subsequently fermented to ethanol. Ho et al. [48] investigated the potential of *C. vulgaris* PS-E as the bioethanol feedstock. This species contains 51% of carbohydrates, which were hydrolyzed through an enzymatic process to give a glucose yield of 0.461 g glucose/g dry biomass. The ethanol yield obtained in their study was 11.7 g/L.

*C. vulgaris* was also reported to be a successful bioremediation agent of palm oil mill effluent (POME), with reductions of ammonia-nitrogen, phosphorus, COD, and biochemical oxygen demand (BOD) of 61, 84, 50.5, and 61.6%, respectively [49]. Bich et al. [50] reported that *C. vulgaris* was used in the treatment of rubber latex concentrate processing wastewater and that this microalga reduced the COD and total Kjeldahl nitrogen (TKN) by 93.4 and 79.3%, respectively. Another study carried out by Nordin et al. [51] used high-rate algal ponds (HRAPs) to treat rubber effluent from an anaerobic digester, and the reductions in COD, BOD, $NH_3$-N, and phosphorous reached 69.1, 87.4, 62.2, and 21.3%, respectively. In the HRAP, *Chlorella* was the predominant genus [51].

Moderately polluted textile wastewater was previously reported to be treated using the microalga *C. vulgaris*, with color and COD reductions of up to 69.9 and 75.7%, respectively [52]. Another study found that this species could degrade 63–69% mono-azo dyes into simple aromatic compounds [53]. Lim et al. [54] investigated the treatment of textile wastewater using 10 different strains of microalgae and found that *C. vulgaris* was able to remove color from the wastewater. When cultured in a HRAP, color removal reached 50% along with high reductions in COD, $PO_4^{3-}$-P, and $NH_4^+$-N [54].

Two wild-type green algae such as *Micractinium* sp. and *Chlorella* sp. can also be grown in high-nitrogen wastewater (mixture of sludge centrate and primary effluent wastewater). The extraction and analysis of extracellular polymeric substances (EPSs) in both algal species during cultivation showed that *Micractinium* generated a higher amount of EPS proteins than *Chlorella* [27]. This fact affects the anaerobic biodegradability and methane yield when these algae are anaerobically co-digested with waste-activated sludge (WAS).

### *2.1.2. Scenedesmus genus*

Food wastewater (FW), rich in nutrients including N, P, Ca, Fe, Al, and total organic carbon (TOC), was also effectively used for microalgal cultivation [9]. The effect of FW supplementation on the biomass and lipid productivity of *Scenedesmus obliquus* cultivated in Bold's Basal Medium (BBM) was recently investigated by Ji et al. [9]. They reported a substantial increase in growth and lipid productivity with supplementation of 1% FW to BBM. Furthermore, the fatty acid methyl ester (FAME) analysis revealed that the palmitic and oleic acid contents increased by up to 8% with the addition of FW. They also noted that FW promoted algal autoflocculation due to the formation of inorganic precipitates at an alkaline pH [9]. Similarly, the

biomass, lipid productivity, and nutrient removal efficiency of *S. obliquus* cultivated under mixotrophic conditions in municipal wastewater were reportedly enhanced when supplemented with FW and flue gas $CO_2$ [55].

Shanab et al. [56] demonstrated that out of three fresh water microalgal isolates selected for heavy metal tolerance studies, *Scenedesmus quadricauda* showed tolerance to heavy metals such as $Hg^{2+}$, $Pb^{2+}$, and $Cd^{2+}$ in up to 100 mg/L concentrations. Research on the applications of immobilized microalgal cells indicated that immobilized algal cells are more tolerant to heavy metal stress when compared to free living cells [56].

*Scenedesmus* sp. has also been widely reported with good carbon sequestration potential [57]. These studies obtained hydrocarbons from microalgal lipids for their conversion into sustainable biofuels as a substitute for fossil hydrocarbons. Furthermore, microalgae have significant applications in the production of valuable materials in the food and pharmaceutical industries, producing a high value-added process in the biosequestration of $CO_2$ [57].

Similar to bioconversion, some microalgae can also carry out the biosorption of textile wastewater. For instance, *S. quadricauda* has been successfully employed as biosorbent to remove remazol brilliant blue R (RBBR) [58, 59].

In a very recent study, microalgae digestate and secondary effluent were used to grow *Scenedesmus* sp. in a tertiary treatment using a 30 L closed photobioreactor for cultivation. The microalgae biomass, composed of *Scenedesmus* sp., reached and maintained a concentration of 1.1 g TSS/L during 30 days [22]. A complete removal of N-$NH_4^+$ and P-$PO_4^{3-}$ and high nitrate and organic matter removals were achieved (58% N-$NO_3^-$ and 70% COD) with 8 days of HRT [22].

### *2.1.3. Dunaliella salina*

A very recent study assessed the feasibility of the cultivation of *D. salina* in controlled environment tertiary-treated municipal wastewater [60]. *D. salina* was selected for its high β-carotene generation capacity and for being a halophilic species to protect our fresh water resources further through wastewater remediation. Nutrient analyses indicated that *D. salina* can significantly remove nitrate, ammonia, and phosphorus from municipal wastewater in the range of 45–88%. Among all combinations studied, optimal algal growth was observed at 30 ppt salinity level, with a 75% wastewater concentration (3:1 ratio of wastewater and saline water mixture, which is the growth medium). These findings concluded that *D. salina* has great capacity for nutrient uptake while providing high-value bioproducts [60].

Another study assessed the production rates of some native microalgae growing in media supplemented with algal digestate, urban wastewater, or digested sludge. Very low production rates, or no growth, were measured when microalgae isolated from high-salinity waters (*D. salina*) were used, suggesting that populations well adapted to extreme environmental conditions are not suitable candidates for growing in wastewater or anaerobic digestate [61].

### *2.1.4. Nannochloropsis salina*

The potential for *N. salina* to be integrated with contaminated water sources was assessed for the concurrent production of a biofuel feedstock while providing an environmental service

through bioremediation [62]. Individual contaminants (As, Cd, Cr, Co, Cu, Pb, Ni, Hg, Se, and Zn) at various concentrations ranging from a low concentration (1X) to higher concentrations (10X and 40X) found in contaminated systems (mine tailings, wastewater treatment plants, produced water) were introduced into growth media. Biological growth experimentation was performed in triplicate at the various contaminant concentrations and at three different light intensities. Results showed that baseline concentrations of each contaminant slightly decreased biomass growth between 89 and 99% of the control with the exception of Ni, which dramatically reduced growth. Increased contaminant concentrations resulted in progressively lower growth rates for all the contaminants tested. Lipid analysis showed that most baseline contaminant concentrations slightly decreased or had minimal effects on lipid content at all light levels. Trace contaminant analysis on the biomass showed that Cd, Co, Cu, Pb, and Zn were sorbed by the microalgae with minimal contaminants remaining in the growth media, which illustrated the effectiveness of microalgae to bioremediate these contaminants when levels are sufficiently low and to not detrimentally impact productivity. The microalgae biomass was less efficient in the sorption of As, Cr, Ni, and Se [62].

Another study revealed that metal levels in municipal wastewaters were unlikely to inhibit algal growth and lipid production at least by metals, which are tolerant to microalgae like *N. salina*. Cells grew without inhibition in treated municipal wastewater or centrate derived from wastewater treatment with the addition of up to 75% v/v in their normal growth medium minus nitrogen and phosphorus [63].

#### *2.1.5. Botryococcus braunii*

*B. braunii* is a microalga, which is regarded as a potential source of renewable fuel because of its ability to produce large amounts of lipids that can be converted into biodiesel. Agro-industrial by-products and wastes are of great interest as cultivation medium for microorganisms because of their low cost, renewable nature, and abundance. Two strategies for the low-cost production of *B. braunii* biomass with high-lipid content were performed: (i) mixotrophic cultivation using molasses, a cheap by-product from the sugar cane plant as a carbon source, and (ii) photoautotrophic cultivation using nitrate-rich wastewater supplemented with $CO_2$ as a carbon source. Mixotrophic cultivation added with 15 g/L molasses produced a high amount of biomass at 3.05 g/L with a high-lipid content of 36.9%. The photoautotrophic cultivation in nitrate-rich wastewater supplemented with 2.0% $CO_2$ produced a biomass of 2.26 g/L and a lipid content of 30.3%. The benefits of this photoautotrophic cultivation are that this cultivation would help to reduce the accumulation of atmospheric carbon dioxide and more than 90% of the nitrate could be removed from the wastewater. When this cultivation was scaled up in a stirred tank photobioreactor and run with the semi-continuous cultivation regime, the highest microalgal biomass of 5.16 g/L with a comparable lipid content of 32.2% was achieved [64].

To understand the potential of using swine lagoon wastewater to cultivate *B. braunii* for biofuel production, the growth characteristics of *B. braunii* 765 cultivated in aerated swine lagoon wastewater (ASLW) without sterilization and pH adjustment were investigated. The results showed that the alga strain could maintain a competitive advantage over the 26-day cultivation. The highest dry biomass of alga grown in ASLW was 0.94 mg/L at Day 24, which was 1.73 times that

grown in a BG 11 medium, an artificial medium normally used for *B. braunii* cultivation. And the algal hydrocarbon content was 23.8%, which was more than twice that in the BG 11 medium. Additionally, after the 26-day cultivation period, about 40.8% of TN and 93.3% of TP in ASLW were removed, also indicating good environmental benefits of algal bioremediation [65].

A study was conducted to evaluate the possibility of using wastewater from a soybean curd manufacturing plant as a growth promoter of *B. braunii* strain BOT-22. Soybean curd wastewater (SCW) was added to a AF-6 medium to set final concentrations at 0 (control), 1, 2, 5, and 10% (v/v). The growth and hydrocarbon production observed in the cultures with 1 and 2% SCW were significantly higher than that observed in the control. It was postulated that proteins and/or reducing sugars in SCW could enhance the growth [66].

#### *2.1.6. Micractinium genus*

The strain *Micractinium* sp. IC-76 was grown in municipal wastewater and showed a biomass productivity of 37.1 ± 4.1 mg/(L d) and a lipid content of 36.2 ± 0.1%, with a total content of saturated and monounsaturated fatty acids of 71.9%. The efficiency of nitrogen ($N\text{-}NH_4^+$) and phosphorus ($P\text{-}PO_4^{3-}$) removal was 96.4 ± 0.7 and 77.8 ± 5.6%, respectively. The strain *Micractinium* sp. IC-76 in the stationary phase of growth showed a significant difference in carbohydrate metabolism, especially sucrose concentration. High-lipid induction during cultivation in wastewater was also driven by changes in the biosynthesis of amino acids, fatty acids, and the tricarboxylic acid cycle [67].

*Micractinium* sp. Embrapa | LBA32 presented vigorous growth in a light-dependent manner in undiluted vinasse under non-axenic conditions. Microalgae strains presented higher biomass productivity in vinasse-based media when compared to standard BBM in cultures performed using 15 L airlift flat plate photobioreactors. Chemical composition analyses showed that proteins and carbohydrates comprise the major fractions of algal biomass. Glucose was the main monosaccharide detected, ranging from 46 to 76% of the total carbohydrate contents according to the culture media used [68].

### 2.2. Haptophytes: *Isochrysis galbana*

A recent study investigates the capacity of *I. galbana* in the bioremediation of aquaculture wastewater from a gray mullet *Mugil cephalus*. The experiment was conducted in batch conditions for 7 days using completely mixed bubble column photobioreactors. After 2 days, *I. galbana* removed 32 and 79% of dissolved inorganic nitrogen and dissolved inorganic phosphorus, respectively [10].

It has been also reported that *I. galbana* cultured in open ponds has fatty acids and a high-protein content, which are suitable for animal nutrition [20].

### 2.3. Cyanobacteria

#### *2.3.1. Arthrospira platensis*

Phosphorus can be recycled from wastewater through microalgal cultivation and provided to crop plants in the form of microalgal biofertilizers. Guldhe et al. [21] reported filamentous

cyanobacteria *A. platensis* cultivated in aquaculture wastewater as algal biofertilizer for the leafy vegetables Arugula (*Eruca sativa*), Bayam Red (*Amaranthus gangeticus*) and Pak Choy (*Brassica rapa* ssp. *chinensis*). In their study, *A. platensis* biomass showed lower amounts of NPK, while amounts of iron, magnesium, calcium, and zinc were found to be higher in algal biomass when compared to chemical fertilizer (Triple Pro 15-15-15).

Microalgae are a rich source of proteins, pigments, and omega fatty acids and thus find application in human and animal feed production. *A. platensis* is one of the dominant species of microalgae used in the health food industry [69].The omega fatty acids from this microalga are used as human food and animal feed supplements. Phang et al. [70] found that the biomass composition of *Arthrospira* cultured in a high-rate algal pond for the treatment of sago starch processing wastewater can be used as high-quality animal feed, especially in the aquaculture industry. During the mentioned treatment of sago processing wastewater using *Spirulina*, COD, $PO_4^{3-}$-P, and $NH_4^+$-N reductions of 94, 93, and 99%, respectively, were achieved [70].

Zainal et al. [71] reported that *A. platensis* was able to treat wastewater containing heavy metals and removed manganese by 84.9%; chromium by 83.8%; arsenic by 71.4%; nickel by 61.9%; zinc by 55%; copper by 52.8%, and iron by 45.1%.

Similar to bioconversion, microalgae could also carry out the biosorption of textile wastewater. For instance, *A. platensis* was used as a biosorbent to remove reactive red 120 (RR-120) from its aqueous solution. It achieved the maximum biosorption capacity of 482.2 mg/g removing 97% RR-120 from the solution [72].

#### *2.3.2. Oscillatoria tenuis*

The performance of *O. tenuis* to remove nitrogen, phosphorus, and COD from secondary effluents of municipal domestic wastewater was investigated in batch experiments. *O. tenuis* had a biomass productivity of 150 mL/(L·d), a removal rate of $NH_4^+$-N of 96.1%, and total phosphorus and COD removal efficiencies of 82.9 and 92.6%, respectively, within 7 days at an aeration rate of 1.0 L/min [73].

At the same time, *O. tenuis* showed its capacity to remove reactive dyes from textile wastewater. This species degraded azo dyes into simple aromatic amines and decolorized dye wastewater [59].

### 2.4. Binary and mixed culture systems

Maintaining the uni-algal system requires a super clean environment, which can be attained under laboratory conditions only. In the outdoor cultivation of microalgae, it is almost impossible to maintain a uni-algal system. If so, it requires a lot of expertise and skills. Moreover, the biomass productivity of the uni-algal system is limited because of suppressed metabolic activity during night time or dark periods. Alternatively, heterotrophic microalgae are used, which are less sensitive to photoperiods, grow fast, and return high-biomass yields. However, a significant amount of $CO_2$ is produced during oxidative metabolism, which remains unused and is released into the environment. This $CO_2$ can be further utilized by employing autotrophic microalgae in the cultivation matrix. Therefore, the concept of a binary culture system arises [38]. Binary culture is considered superior to the uni-algal system in several

different ways: binary culture can use wastewater as a nutrients source without sterilization unlike in single systems; microalgae observe a low level of contamination in binary culture because bacteria protect those invading pathogens; microalgae with increased growth rate would decrease the cultivation time and reduce the overall cost; binary culture also aids in bioflocculation and lipid induction; and so on [38].

Species selection is crucial for the success of microalgae cultivation in wastewater. Combining different species with varying metabolic potential would provide robustness to fluctuations in environmental factors and wastewater compositions, thereby giving more stability to the system. For instance, the potential application of microalgae consortia (*Chlorella* sp., *Scenedesmus* sp., and *C. zofingiensis*) compared to monoculture (*Chlorella* sp.) for the treatment of dairy wastewater was evaluated by Qin et al. [74]. They reported a significantly higher COD removal (57–62%) and phosphorous removal (91–96%) by microalgae consortia when compared to the monoculture of *Chlorella* sp. Furthermore, FAME profiles indicated that lipids produced from the microalgae consortia cultivation system were more suitable for biodiesel production [74].

In a very recent study [8], a mixed microalgae consortium (highly dominated by *Chlorella* species and small portions of *Scenedesmus* sp.) was cultivated using digestate (D), animal manure (AM), and textile wastewater (TW) as growth medium providing mainly N (nitrogen) and P (phosphorous) sources without any extra nutrient addition. After a cultivation period of 13 days, P was completely removed (100%); however, N was still remaining, and the removal rates of 70.1, 72.3, and 16.7% for TW, AM, and D, respectively, were achieved. The peak growth rate and biomass production of 0.419 $d^{-1}$ and 0.4 g/L (in terms of volatile solids, VSs) were achieved using TW as growth medium [8].

## 3. Use of microalgae for biogas production through anaerobic digestion

Anaerobic digestion is a series of biological processes in which microorganisms break down biodegradable material in the absence of oxygen. The end-products of anaerobic digestion are biogas and a digestate. Recently, algal biomass has been identified and developed as a renewable fuel source, and the growth of algal biomass for methane production has been increased.

The first study concerning the anaerobic digestion of microalga was carried out by Goluke et al. [30]. *Scenedesmus* sp. and *Chlorella* sp. were used as substrates for anaerobic digestion under different conditions. The authors finally concluded that microalgae have a relatively low digestibility due to the slowly biodegradable cell wall. Recently, one of the first studies about using algal biomass in anaerobic digestion was carried out by De Schamphelaire et al. [75]. This work consisted of designing a closed loop where algal biomass was used to obtain biogas. The maximum methane yield reached was 65 mL/day. More recently, in 2013, Torres et al. [35] defined the ideal microalgae for anaerobic digestion as a large cell microalga with a very thin cell wall or lacking it, with a high-growth rate in non-sterile medium and great resistance against natural pollutants. In one of the latest studies on the anaerobic digestion of microalgae, the authors pointed out the main limitations during the anaerobic digestion of

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microalgae, noting the low degradability of the cell wall, ammonium toxicity, and salinity as the main inhibitors of anaerobic digestion [76].

However, the use of microalgae as co-substrate is an approach to dilute complex compounds and balance the C/N ratio. Co-digestion has several advantages such as adjusting the C/N ratio, nutrients, and inhibitor compounds [34]. Ajeej et al. [77] also reported the increased activity of methanogenic microorganisms, a decreased anaerobic digestion inhibition by ammonium, and even increased cellulose activity when carbon-rich materials were added. Taking into account that the C/N ratio of the microalgal biomass is around 10:1 [78], the microalgae biomass can be considered as a suitable feedstock for carbon-rich substrates [79].

The main microalgae used for co-digestion have been described in the following paragraphs.

### 3.1. Chlorophytes

#### *3.1.1. Chlorella genus*

Ehimen et al. [80] added lipid-extracted *Chlorella* biomass resulting from microalga diesel production to glycerol (main by-product formed during the transesterification process) and observed an increase in the methane yield of 50% when compared to the digestion of residual biomass alone.

Wang et al. [81] used the biomass of microalga *Chlorella* sp. grown in laboratory culture for co-digestion with WAS. The batch experiments were carried out under mesophilic conditions with a working volume of 100 mL. Different volumes of algae and WAS were added to the digester. They experimentally proved that the addition of WAS improved the anaerobic digestion of the microalga *Chlorella*, producing 73–79% more methane than single microalga digestion. Similar results were obtained by Li et al. [82], who co-digested *Chlorella* sp. with chicken manure in batch experiments. The co-digestion enhanced the methane production obtained during the single digestion of chicken manure and *Chlorella* sp. by 14.20 and 76.86%, respectively. By contrast, Retfalvi et al. [83], using the same C/N ratio, but pretreating the microalga, did not observe any positive effects on methane production.

Beltran et al. [84] assessed the co-digestion of *C. sorokiniana* with WAS. Different co-digestion mixtures were tested in biochemical methane potential (BMP) tests under mesophilic conditions. The highest methane yield obtained was 442 mL $CH_4$/g VS for the mixture 75% WAS and 25% microalga. This value was 22 and 39% higher than that obtained in the anaerobic digestion of the sole substrates, WAS and microalga, respectively. This mixture clearly improved anaerobic digestion by ensuring its viability, suitability, and efficiency.

Rusten and Sahu [85] co-digested *Chlorella* sp. biomass and wastewater sludge (pretreated sludge liquor).The specific methane gas production (mL $CH_4$/g $VS_{fed}$) was not increased when compared to the anaerobic digestion of wastewater sludge alone. The co-digestion process achieved between 65 and 90% of specific methane gas production for sludge liquor depending on the HRT, temperature of incubation, and pretreatment of algae biomass. However, this study indicated that tested microalga could be cultivated in reject water to remove nitrogen and phosphorus from the sludge liquor.

In a recent study, Mahdy et al. [86] investigated the anaerobic co-digestion of *C. vulgaris* and manure. They used five different mixtures in a batch mesophilic experiment. The percentage 80:20 microalga:manure produced 431 mL $CH_4$/g VS, while the methane yield of the single microalga produced 415 mL $CH_4$/g VS. Despite the high-ammonium levels (3.7–4.2 g $NH_4^+$-N/L), using ammonia tolerant inoculums resulted in a relatively high-methane yield.

According to Li et al. [82], *Chlorella* 1067 was cultivated in a chicken manure-based digestate, and the resulting algae biomass was used as co-substrate with chicken manure in anaerobic co-digestion. The growth of microalga in manure-based digestate recycled about 91% of the total nitrogen and 86% of the soluble organic phosphorous. During co-digestion, the highest methane production was 238.71 mL $CH_4$/g VS, obtained at the mixing ratio of 8:2 (chicken manure to *Chlorella* 1067 according to the VS).

### *3.1.2. Scenedesmus genus*

Ramos-Suarez et al. [87] described *Scenedesmus* sp. biomass as a non-suitable substrate for anaerobic digestion due to its low degradability and low methane production. In contrast, during their investigations, they used the biomass of microalga as co-substrate with *Opuntia maxima* cladodes. Bioreactors were used to grow *Scenedesmus* sp., and the biomass was co-digested with different percentages of cladodes of 1 or 2 years of age in order to avoid an increase in lignocelluloses. C/N ratios from 6.0 to 51.3 were used, proving that co-digestion improved methane yield and kinetics when compared to the mono-digestion of both substrates. The best mixture turned out to be the C/N ratio of 15.6. The methane yield for this mixture was 233.6 ± 16.4 mL $CH_4$/g VS and was increased by 66.4 and 63.9% when compared to *Scenedesmus* sp. biomass and *O. maxima*, respectively, when digested alone.

Astals et al. [88] assessed the co-digestion of pig manure and *Scenedesmus* sp. with and without the extraction of intracellular algal co-products. Proteins and/or lipids were extracted from *Scenedesmus* sp. This process increased methane yield by 29–37% when compared to raw microalga biomass. Co-digestion experiments showed a synergy effect between pig manure and raw microalga that increased raw algae methane yield from 163 to 245 mL $CH_4$/g VS. A similar synergy effect was not observed when algal residues were co-digested with pig manure.

Arias et al. [22] used microalga digestate and secondary effluent to grow microalga in a tertiary wastewater treatment, and then the microalga biomass was co-digested for biogas generation. The algal biomass was mainly composed of *Scenedesmus* sp. The algae biomass and the WAS were pretreated by autohydrolysis reaching 11.4 and 25.7% of solubilization, respectively. The solubilization of *Scenedesmus* biomass was lower than the solubilization of WAS after pretreatment, and *Scenedesmus* has been reported to have a complex multilayer cell wall [89]. After pretreatment both substrates were co-digested in different proportions. The maximum methane yield obtained was 204 mL $CH_4$/g VS for the anaerobic digestion of 100% WAS. On the other hand, the methane yield of the anaerobic digestion of 100% microalga exhibited a 64% lower methane production and reached 134 mL $CH_4$/g VS. The mixture of 20% microalga and 80% WAS produced 187 mL $CH_4$/g VS, while the mixture of 50% microalgae and 50% WAS produced 162 mL $CH_4$/g VS, and the mixture of 80% microalga and 20% WAS produced

132 mL $CH_4$/g VS. The results showed neither positive nor negative synergies between substrates, meaning that co-digestion did not improve microalga anaerobic biodegradability [22].

### *3.1.3. Dunaliella salina*

According to Fernández-Rodríguez et al. [36], the addition of olive mill solid waste (OMSW) to *D. salina* biomass resulted in the improvement in methane yield and biodegradability of OMSW when compared to the anaerobic digestion of the sole substrates. The experiment was carried out in batch, and different percentages of OMSW and *D. salina* biomass were tested. The highest biodegradability was found for the co-digestion mixture of 50% OMSW and 50% *D. salina*. Nevertheless, the maximum methane production, 330 mL $CH_4$/g VS, and the highest methane production rate were obtained for the co-digestion mixture of 75% OMSW and 25% *D. salina*, keeping a C/N ratio close to 26.7.

### *3.1.4. Nannochloropsis salina*

Another approach to enhance biogas production from microalga through co-digestion was assessed by Schwede et al. [90]. Corn silage is one is the most common waste products produced around all over the world. Corn silage is characterized as being a lignocellulosic residue and very difficult to digest by anaerobic digestion [91]. The experiment carried out by Schwede et al. [90] reached a high-methane yield using *N. salina* as a co-substrate of corn silage. The mixture balanced the nutrient composition due to the corn silage providing mainly carbon and the microalga providing nitrogen, which helped to balance the C/N ratio from 65 (*N. salina*) or 32.6 (corn silage) to 21.2 (Mixture *N. salina*/corn silage). This mixture, C/N = 21.2, reached 9% more methane than that obtained in the anaerobic digestion of the corn silage alone.

### *3.1.5. Botryococcus braunii*

Neumann et al. [92] reported that the anaerobic co-digestion of lipid-spent *B. braunii* (LSBB) with WAS and glycerol showed no significant increase in BMP when mixing these substrates. However, the kinetic constant of the mixture 25% WAS-75% LSBB was much higher than those obtained for WAS and LSBB alone. The mixture of 10% glycerol and 90% LSBB did not show a higher kinetic constant or methane production. The authors concluded that the application of different cultivation procedures, lipid extraction methods, and anaerobic conditions will result in different microalga biomass compositions and characteristics, which affect the productivity of microalgal methane.

### *3.1.6. Micractinium genus*

Wang et al. [27] applied WAS to the digestion of microalga biomass consisting of *Micractinium* sp. The algae biomass was grown in high-nitrogen wastewater (mixture of sludge centrate and primary effluent wastewater). The microalga showed a good ability for nutrient removal throughout the growth. The co-digestion of microalga biomass and WAS improved the solubilization efficiency and the biodegradability of the microalgae. The methane yield obtained

for the microalga was 209 mL/g VS. The co-digestion of algae with WAS improved the volatile solid reduction, the solubilization efficiency of the algae, and their biogas yield. However, the methane production of the WAS alone showed no improvement.

*3.1.7. Selenastrum capricornutum (Chlorophyta) and Isochrysis galbana (Haptophyta)*

*I. galbana* and *S. capricornutum* were co-digested with sewage sludge under mesophilic (33°C) and thermophilic (55°C) conditions [93]. Under mesophilic conditions, the anaerobic digestion of sewage sludge produced 451 ± 12 mL biogas/g VS. The microalga *I. galbana* produced 439 mL biogas/g VS, and *S. capricornutum* produced 271 mL biogas/g VS. When a substrate mixture was fed, biogas production showed quite similar values for all experiments, regardless of the sludge to microalga ratio in the mixture. The average biogas production was 440 ± 25 mL biogas/g VS. So, microalga and sewage sludge co-digestion did not improve biogas yield in comparison with individual digestions of both substrates under mesophilic conditions. Under thermophilic conditions, the biogas production of *I. galbana* was 261 ± 11 mL biogas/g VS, and the production of *S. capricornutum* was 185 ± 7 mL biogas/g VS. The amount of methane decreased by 40.5 and 31.7% for *I. galbana* and *S. capricornutum*, respectively, when compared to their biogas production at 33°C. The increase in temperature had a negative influence on microalga digestion. However, temperature had a huge beneficial effect on sewage sludge. The production of biogas reached 566 ± 5 mL biogas/g VS, indicating that 25.5% more biogas was produced by increasing temperature. The experiment presented similar tendencies, the higher the volatile solid, the lower the biogas production.

**3.2. Cyanobacteria**

*3.2.1. Arthrospira platensis*

*A. platensis* was characterized as having a high level of protein and, therefore, a high-nitrogen content [94]. Biomass with a high-nitrogen content could be used as co-substrate with high-carbon content substrates [95]. This study investigated the co-digestion of *A. platensis* with barley straw, beet silage, and brown seaweed at a C/N ratio of 25, the optimal ratio for anaerobic digestion [96]. The experiments were carried out in batch and semi-continuous systems. The C/N ratios of the substrates were 4.3, 145.5, 41.7, and 28.7 for *A. platensis*, barley straw, beet silage, and seaweed *Laminaria digitata*, respectively. The methane productions during the batch experiments were 357.1, 196.8, 393.5, and 306.5 $mL_N$/gVS for *A. platensis*, barley straw, beet silage, and seaweed *L. digitata*, respectively. The co-digestion of 45% *A. platensis* and 55% beet silage produced 360.9 $mL_N$/gVS. The co-digestion of 85% *A. platensis* and 15% barley straw produced 347.8$mL_N$/gVS, and the best co-digestion mixture of *A. platensis* and *L. digitata* (15–85%) produced 311.5 $mL_N$/gVS. Mono-digestion of *A. platensis* led to high-methane yields in the semi-continuous mode but only at low-organic rates of 1.0 g VS/L·d. Co-digestion with carbon-rich substrates had a positive effect on process stability. The highest biogas production occurred during co-digestion of microalga with beet silage. The best process stability was found at an organic loading of 4.0 g VS/L·d during co-digestion with the seaweed *L. digitata* [95].

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*A. platensis* was co-digested with WAS in batch and in semi-continuous systems [97]. During the batch tests, the system reached 89–93% volatile solid reduction. The biogas production was between 210 and 260 mL $CH_4$/g VS. In the continuous studies, a two-phase anaerobic digestion system was investigated. The system achieved 60% of volatile solid reduction with 525 mL biogas/gVS·d. The co-digestion of *A. platensis* and sewage sludge improved biogas production and volatile solid reduction. The best mixture was 66.6% WAS and 33.3% *A. platensis* based on volatile solids. The maximum methane production was 640 mL biogas/g VS·d with a 62.5% reduction in volatile solids. The methane content in the biogas was 77%.

#### *3.2.2. Oscillatoria tenuis*

Cheng et al. [73] carried out batch experiments to investigate the performance of *O. tenuis* to remove nitrogen, phosphorus, and COD and from the secondary effluents of municipal domestic wastewater. The potential of biogas production was also investigated by applying the co-digestion of *O. tenuis* with pig manure. *O. tenuis* had a good biomass productivity, which ranged from 104 to 150 mg/L·d, and was beneficial for the subsequent anaerobic digestion. A maximum methane yield of 191 mL $CH_4$/g VS was achieved through co-digestion of this microalga with pig manure at a mixing ratio of 2.0.

### 3.3. Binary culture system

#### *3.3.1. Scenedesmus genus and Chlorella genus*

Zhen et al. [98] used a mixed microalgae culture of *Scenedesmus* sp. and *Chlorella* sp., which were co-digested with food waste in a batch system under mesophilic conditions. The results showed that supplementing food waste with microalga significantly improved the performance of microalga digestion. The highest methane yield achieved was 639.8 ± 1.3 mL/g VS, which was reached at a microalga/food waste ratio of 0.2:0.8, obtaining a 4.99-fold increase with respect to microalgae alone (106.9 ± 3.2 mL/g VS).

#### *3.3.2. Microalgae and bacteria*

Solé-Bundó et al. [99] grew microalgae biomass in wastewater, and subsequently, the algae-bacteria biomass was co-digested with wheat straw. Batch systems were used for testing different substrate percentages (20–80%, 50–50% and 80–20%, microalgae and wheat straw, respectively, on a volatile solid basis). The highest synergies in degradation rates were observed by adding at least 50% wheat straw. Therefore, the co-digestion of 50% microalgae biomass and 50% wheat straw was further investigated in mesophilic semi-continuous lab-scale reactors. The results showed that the methane yield was increased by 77% in the co-digestion when compared to microalgae biomass mono-digestion.

**Table 1** summarizes the different microalgae and co-substrates tested in anaerobic co-digestion processes including the improvement in the methane yields observed.

| Microalga | Co-substrate | Conditions | Improvement in methane yield (%) | Reference |
|---|---|---|---|---|
| Lipid-extracted *Chlorella* biomass | Glycerol C/N = 12.44 | Laboratory scale, continuously stirred tank reactor, at mesophilic temperature | >50 (compared to microalga) | [80] |
| *Chlorella* sp. (4%) | WAS (96%) | Batch at mesophilic temperature | 73–79 (compared to microalga) | [81] |
| *Chlorella* 1067 (20%) | Chicken manure (80%) | Batch experiments | 77 (compared to microalga) | [82] |
| Pretreated *Chlorella* sp. (80%) | Chicken manure (20%) | Batch experiments | No positive effect | [83] |
| *C. sorokiniana (25%)* | WAS (75%) | Batch at mesophilic temperature | 39 (compared to microalga) | [84] |
| *Chlorella* sp. (12%) | Wastewater sludge (88%) | Batch at mesophilic temperature | 12 (compared to single substrate) | [85] |
| *C. vulgaris (80%)* | Manure (20%) | Batch at mesophilic temperature | 3.8 (compared to microalga) | [86] |
| *Scenedesmus* sp. (25%) | *O. maxima* cladodes (75%) | Batch at mesophilic temperature | 66.4 (compared to microalga) | [87] |
| *Scenedesmus* sp. (15%) | Pig manure (85%) | Batch at mesophilic temperature | 50.3 (compared to microalga) | [88] |
| *Scenedesmus* sp. (20%) | WAS (80%) | Batch at mesophilic temperature | 39.5 (compared to microalga) | [22] |
| *D. salina (25%)* | OMSW (75%) | Batch at mesophilic temperature | 3 (compared to single substrate) | [36] |
| *N. salina (16.6%)* | Corn silage (83.4%) | Batch at mesophilic temperature | 6 (compared to microalga) | [90] |
| Lipid-spent *B. braunii* | WAS and glycerol | Batch at mesophilic temperature | No positive effect | [92] |
| *Micractinium* sp. (79%) | WAS (21%) | Batch at mesophilic temperature | 10 (compared to microalga) | [27] |
| *I. galbana* and *S. capricornutum* | Sewage sludge | Batch at mesophilic and thermophilic temperature | No positive effect | [93] |
| *A. platensis* (85%) | Barley straw (15%) | Batch at mesophilic temperature | 76.7 (compared to single substrate) | [95] |
| *A. platensis* (45%) | Beet silage (55%) | Batch at mesophilic temperature | 1.1 (compared to microalga) | [95] |
| *A. platensis* (15%) | *L. digitata* (85%) | Batch at mesophilic temperature | 1.6 (compared to single substrate) | [95] |
| *A. platensis* (33.3%) | WAS (66.6%) | Two stages semi-continuous | 32.5 (compared to microalga) | [97] |
| *O. tenuis* (66.6%) | Pig manure (33.3%) | Batch at mesophilic temperature | * | [73] |

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| Microalga | Co-substrate | Conditions | Improvement in methane yield (%) | Reference |
|---|---|---|---|---|
| *Scenedesmus* genus + *Chlorella* genus (20%) | Food waste (80%) | Batch at mesophilic temperature | 498.5 (compared to microalga) | [98] |
| *Chlorella* sp. + some *Monoraphidium* sp. (50%) | Wheat straw (50%) | Batch at mesophilic temperature | 77 (compared to microalga) | [99] |

C, carbon; N, nitrogen; WAS, waste-activated sludge; OMSW, olive mill solid waste; *, not available.

**Table 1.** Improvement of methane yields after anaerobic co-digestion processes of microalgae with different substrates.

## 4. Microalgae growth in anaerobic digestates

### 4.1. Physico-chemical characterization of digestates

The anaerobic digestate studied by Solé-bundó et al. [100] presented low dry matter content (~3%), and these digestates can therefore be treated as liquids that could be directly spread onto soil as fertilizer. A problem arises when transportation is required and moisture reduction could be necessary. Anaerobic digestate from microalgae co-digestion was observed to present better water release than the digestate from single microalga digestion.

Other parameters that could have a negative impact on soil (pH, electrical conductivity, and volatile fatty acids) were lower in the co-digestion digestates, indicating that microalgae co-digestion resulted in a more stable digestate.

In general, among the bibliography, anaerobic digestates from agro-food industries presented higher organic contents than those from microalgae digestion [101], which could be explained due to organic matter mineralization during anaerobic digestion processes. The use of microalgae as co-substrate in the digester reduces the VS/TS ratio when compared to microalga alone (from 53 to 54–47%) due to the better biodegradability of the organic compounds of the co-substrate.

In order to evaluate the feasibility of these anaerobic digestates as fertilizers, some elemental nutrients were evaluated. The total nitrogen content was higher in the non-co-digested microalgae (80 g/kg TS and 56 g/kg TS), although the $N\text{-}NH_4^+$/TKN ratio, which represents the soluble mineral nitrogen fraction, only varied from 30.9 to 33.8% among all digestates. Moreover, the C/N ratio was low across the board, which means that in each case the nitrogen content is too high for its use as fertilizers, although it could be used as soil amendment. This problem could be sorted out by using a high-carbon content co-substrate like OMSW or corn silage. Phosphorous and potassium were found slightly higher in the digestates from non-co-digestion, although in each case the content was relatively low and similar to other anaerobic digestates reported in the literature. Calcium, magnesium, and sodium were also analyzed, and no difference was observed among the different digestates [100].

On the whole, the anaerobic digestate from microalgae co-digestion presented better suitability for nutrient supply in soil due to its low C/N ratio, which could be enhanced by using a co-substrate with a higher carbon content.

### 4.2. Microalgae growth in digestates

The anaerobic digestion of biomass produces a high-nutrient digestate, which is usually used as crop fertilizer, and also could be used as a nutrient supply for microalgae growth in order to reduce the use of external sources of nitrogen and phosphorous [102]. Moreover, wastewaters and other biomass present a reduction in suspended solids and color, better degradability, a more stable pH, and a reduction in pathogens after the anaerobic digestion process, which could enhance microalgae growth when compared to the non-digested biomass.

The main factors that could affect the microalgae growth in anaerobic digestates are the nitrogen and phosphorous contents as well as the pH profile. pH could be increased due to active photosynthesis or insufficient $CO_2$ supply, which could provoke a $N\text{-}NH_4^+$ disappearance through gas stripping and a $P\text{-}PO_4^{3-}$ precipitation when the medium presents a high concentration of $Ca^{2+}$ [103]. Thus, when the pH of the medium is increased due to microalgae activity, nitrogen and phosphorous depletion do not necessarily mean an increase in biomass. Moreover, it has been reported that an ammonia concentration higher than 2 mM, when pH exceeds 8.1, presented a toxic effect on algae growth [104]. Regarding phosphorous content, it has been reported that 5 mg P/L was sufficient for adequate algae growth when the N/P ratio was around 15, although other studies suggested that N should be the limiting factor [103].

On the other hand, the organic load in these anaerobic digestates is reduced after microalgae cultivation. Nitrogen and phosphorous could be completely removed when the conditions are optimum and COD reduction could reach 44–85% depending on culture conditions and microalgae species [103].

#### *4.2.1. Chlorophytes*

##### *4.2.1.1. Chlorella genus*

An early study used different microalgae cultivated in swine manure anaerobic digestate diluted with tap water (0.6–3.0%) in order to evaluate its effect on microalgae growth. *Chlorella* sp. was the only species that presented pH stability (pH = 8.5 during 8 days), which indicated that the nitrogen removal was directly related to biomass production. Regarding temperature conditions, *Chlorella* sp. did not show any difference in biomass yield when the temperature was raised from 10 to 20°C. COD reduction in the anaerobic digestate reached 60%. The best conditions for the highest concentration (41 mg dry wt/L·d) were 20°C and a manure concentration of 2% [103].

##### *4.2.1.2. Parachlorella kessleri*

*P. kessleri* was cultivated (12 days; 25°C; air flow: 0.5–1 L/min; illumination: 200 μmol/m²·s) in the anaerobic digestate derived from the co-digestion of end-of-life dairy products with a given mixture of agro-industrial wastes [107]. Prior to the growth of algae, the anaerobic digestate was filtered, diluted (2–10%), and then split into two different samples, one sterilized and the other not. Under the best conditions (2% dilution), *P. kessleri* presented a biomass yield of 270 mg/L, regardless of the use of sterilized or non-sterilized anaerobic digestate.

Moreover, according to the nutrient removal, the nitrogen depletion (up to 100%) and the phosphorous reduction (93.4%) were higher when the anaerobic digestate was sterilized and diluted by up to 2%. Nevertheless, the maximum COD removal (33.3%) was achieved with the non-sterilized anaerobic digestate and a higher dilution (10%). Regarding the fatty acid accumulation, after 25 days of growth, the concentration observed (31.1% dry weight) was higher than in the control essay (19.6% dry weight).

#### *4.2.1.3. Scenedesmus genus*

De la Noüe et al. [103] studied the growth of different microalgae in swine manure anaerobic digestate diluted with tap water (0.6–3.0%). The results showed that *Scenedesmus obliquus* presented a response to high temperature, which could be a problem for outdoor work. This microalga was able to reduce the COD content of the anaerobic digestate by up to 85% with a microalga concentration of 57 mg dry wt/L·d at 20°C and with a manure concentration of 2% after 15 days.

In a different study, *S. obliquus* was cultivated in the abovementioned conditions [107]. Under the best conditions (2% dilution), *S. obliquus* presented a biomass yield of 231 mg/L, regardless of the use of sterilized or non-sterilized anaerobic digestate. Moreover, according to the nutrient removal, the nitrogen depletion was higher (up to 100%) when the anaerobic digestate was sterilized and diluted by up to 2%. Nevertheless, the phosphorous reduction was higher (92.5%) when the anaerobic digestate was not sterilized, and the maximum COD removal (53.7%) was achieved with the non-sterilized anaerobic digestate and a higher dilution (10%). The fatty acid accumulation (26.6% dry weight) was higher after 25 days of growth than in the control essay (24.5% dry weight).

Different anaerobic digestates from microalgae biomass co-digestion with swine and cow manure and vegetable wastes were selected for the growth of *Scenedesmus* sp. AMDD at 22°C [102]. Nitrogen was adjusted to 1.5 mM ($NH_3$-N) with deionized water, and different phosphorous concentrations were evaluated. Moreover, digestates were filtered to reduce the bacterial load. This study showed that the use of an anaerobic digestate from the co-digestion of microalgae biomass presented a good microalga growth rate. Animal manure digestate without co-digestion did not produce a complete nitrogen removal, which was improved when $Mg^{+2}$ was added in the media growth. This element was indicated as a key nutrient for microalgae growth, and it was concluded that 0.03 ± 0.02 mM was adequate for optimal growth.

#### *4.2.1.4. Micractinium pusillum*

*M. pusillum* was grown in a cheese factory anaerobic digestate at 20°C and proven to present a satisfactory microalga growth rate. After 4 days, the pH reached 8.5, and the ammonia depletion was complete, although, according to the high pH, it could be due to the stripping of ammonia or bacterial activity. P-$PO_4^{3-}$ removal reached 33%, and the biomass yield was 137 ± 21 mg dry wt/L·d. Moreover, it was observed that the presence of suspended organic matter caused cell clogging and the adhesion of *M. pusillum* to the walls of the culture vessels [105].

#### *4.2.2. Cyanobacteria*

##### *4.2.2.1. Phormidium bohneri*

De la Noüe et al. [103] also studied the growth of *P. bohneri.* The nitrogen toxic effect for *P. bohneri* was observed at 3.2 mM N-$NH_4^+$, which indicated that *P. bohneri* presented a higher nitrogen resistance than other common cyanobacteria. Moreover, an increase in temperature (from 10 to 35°C) produced an increase in biomass production. It was observed that a concentration of 0.1–0.5 mg $Cu^{2+}$/L showed a toxic effect on *P. bohneri.* Seventy-five percent of COD removal from the anaerobic digestate was achieved. The higher concentration of *P. bohneri* (32 mg dry wt/L·d) was reached with a 2% swine manure dilution at 20°C.

When *P. bohneri* was cultivated in a cheese factory anaerobic digestate at 20°C, a rapid increase in pH was observed after 4 days (from 8.4 to 10.9). No significant amount of $NH_4^+$ was observed after the process, although, according to the high pH, it could be due to the stripping of ammonia or bacterial activity. P-$PO_4^{3-}$ removal reached 69% with a biomass yield of 329 ± 24 mg dry wt/L·d [105].

##### *4.2.2.2. Spirulina maxima*

In an early study, *S. maxima* was observed to need a high concentration of bicarbonate ions for optimal growth [106]. When it was cultivated in swine manure anaerobic digestate diluted with seawater, an increase in the microalga growth rate was observed with $CO_2$ supplementation. After 15 days, the anaerobic digestate presented a complete N-$NH_4^+$ reduction, phosphate removal of 99.3%, nitrogen depletion of 76%, and a reduction in volatile solids of 28%.

## 5. Conclusions

Microalgae are renowned as a powerful biotechnology platform for the production of a wide range of value-added products. These include biofuels, animal and aquaculture feeds, and high-value commercial products, such as pigments, polysaccharides, bioplastics, and other organic compounds. Microalgae have also been proposed for a biorefinery model where multiple compounds can be produced simultaneously from harvested microalgal biomass grown in wastewaters and in anaerobic digestion digestates. The growth of the biomass in industrial wastewater and/ or anaerobic digestates has been proven to be a feasible alternative to synthetic mediums.

Regarding the anaerobic digestion of microalgae and cyanobacteria biomass, co-digestion allows to improve the low C/N ratio of microalgae and cyanobacteria, balance the nutrients, and avoid the possible inhibitions in many cases. Furthermore, the produced digestate after the anaerobic digestion process presented better stability when a high-carbon biomass is co-digested with microalgae or cyanobacteria biomass.

However, the wide variety of microalgae and cyanobacteria and the different types of high-carbon biomass make it difficult to ascertain a general assessment about the enhancement of methane production when these two biomasses are co-digested. In this respect, it seems that the use of microalgae/bacteria consortium could reduce drawbacks from working with

pure species by favoring positive synergetic effects. Further studies will be needed in order to obtain a proper mixture culture.

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## References

[1] Santos-Ballardo DU, Rossi S, Reyes-Moreno C, Valdez-Ortiz A. Microalgae potential as a biogas source: Current status, restraints and future trends. Reviews in Environmental Science and Biotechnology. 2016;**15**:243-264. DOI: 10.1007/s11157-016-9392-z

[2] Najafi G, Ghobadian B, Tavakoli T, Yusaf T. Potential of bioethanol pro-duction from agricultural wastes in Iran. Renewable and Sustainable Energy Reviews. 2009;**13**(6-7): 1418-1427. DOI: 10.1016/j.rser.2008.08.010

[3] MacIntyre HL, Kana TM, Anning T, Geider RJ. Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. Journal of Phycology. 2002;**38**(1):17-38. DOI: 10.1046/j.1529-8817.2002.00094.x

[4] Laws EA, Taguchi S, Hirata J, Pang L. Optimization of microalgal production in a shallow outdoor flume. Biotechnology and Bioengineering. 1988;**32**(2):140-147. DOI: 10.1002/bit.260320204

[5] Geider RJ. Light and temperature dependence of the carbon to chlorophyll a ratio in microalgae and cyanobacteria: Implications for physiology and growth of phytoplankton. The New Phytologist. 1987;**106**:1-34. DOI: 10.1111/j.1469-8137.1987.tb04788.x

[6] Jankowska E, Sahu AK, Oleskowicz-Popiel P. Biogas from microalgae: Review on microalgae's cultivation, harvesting and pretreatment for anaerobic digestion. Renewable and Sustainable Energy Reviews. 2017;**75**:692-709. DOI: 10.1016/j.rser.2016.11.045

[7] Yang J, Xu M, Zhang X, Hu Q, Sommerfeld M, Chen Y. Life-cycle analysis on biodiesel production from microalgae: Water footprint and nutrients balance. Bioresource Technology. 2011;**102**(1):159-165. DOI: 10.1016/j.biortech.2010.07.017

[8] Huy M, Kumar G, Kim H-W, Kim S-H. Photoautotrophic cultivation of mixed microalgae consortia using various organic waste streams towards remediation and resource recovery. Bioresource Technology. 2018;**247**:576-581. DOI: 10.1016/j.biortech.2017.09.108

[9] Ji MK, Yun HS, Park S, Lee H, Park YT, Bae S, Ham J, Choi J. Effect of food wastewater on biomass production by a green microalga *Scenedesmus obliquus* for bioenergy generation. Bioresource Technology. 2015;**179**:624-628. DOI: 10.1016/j.biortech.2014.12.053

[10] Andreotti V, Chindris A, Brundu G, Vallainc D, Francavilla M, Garcia J. Bioremediation of aquaculture wastewater from *Mugil cephalus* (Linnaeus, 1758) with different microalgae species. Chemistry and Ecology. 2017;**33**:750-761. DOI: 10.1080/02757540.2017.1378351

[11] Ryther JH, Dunstan WM. Nitrogen, phosphorous, and eutrophication in the coastal marine environment. Science. 1971;**171**(3975):1008-1013. DOI: 10.1126/science.171.3975.1008

[12] Li R, Duan N, Zhang Y, Liu Z, Li B, Zhang D, Lu H, Dong T. Co-digestion of chicken manure and microalgae *Chlorella* 1067 grown in the recycled digestate: Nutrients reuse and biogas enhancement. Waste Management. 2017;**70**:247-254. DOI: 10.1016/j.wasman.2017.09.016

[13] Thorin E, Olsson J, Schwede S, Nehrenheim. Co-digestion of sewage sludge and microalgae – Biogas production investigations. Applied Energy. 2017. In Press. DOI: 10.1016/j.apenergy.2017.08.085

[14] Razzak SA, Ali SAM, Hossain MM, deLasa H. Biological $CO_2$ fixation with production of microalgae in wastewater – A review. Renewable and Sustainable Energy Reviews. 2017;**76**:379-390. DOI: 10.1016/j.rser.2017.02.038

[15] Hirata S, Hayashitani M, Taya M, Tone S. Carbon dioxide fixation in batch culture of *Chlorella* sp. using a photobioreactor with a sunlight-collection device. Journal of Fermentation and Bioengineering. 1996;**81**:470-472. DOI: 10.1016/0922-338X(96)85151-8

[16] Maeda K, Owada M, Kimura N, Omata K, Karube I. $CO_2$ fixation from the flue gas on coal-fired thermal power plant by microalgae. Energy Conversion and Management. 1995;**36**:717-720. DOI: 10.1016/0196-8904(95)00105-M

[17] Kao C-Y, Chen T-Y, Chang Y-B, Chiu T-W, Lin H-Y, Chen C-D, Chang J-S. Utilization of carbon dioxide in industrial flue gases for the cultivation of microalga *Chlorella* sp. Bioresource Technology. 2014;**166**:485-493. DOI: 10.1016/j.biortech.2014.05.094

[18] Brown N, Shilton A. Luxury uptake of phosphorus by microalgae in waste stabilization ponds: Current understanding and future direction. Reviews of Environmental Science Biology and Biotechnology. 2014;**13**:321-328. DOI: 10.1007/s11157-014-9337-3

[19] Rahman A, Putman RJ, Inan K, Sal FA, Sathish A, Smith T, Nielsen C, Sims RC, Miller CD. Polyhydroxybutyrate production using a wastewater microalgae based media. Algal Research. 2015;**8**:95-98. DOI: 10.1016/j.algal.2015.01.009

[20] Udaiyappan AFM, Hasa HA, Takriff MS, Abdullah SRS. A review of the potentials, challenges and current status of microalgae biomass applications in industrial wastewater treatment. Journal of Water Process Engineering. 2017;**20**:8-21. DOI: 10.1016/j.jwpe.2017.09.006

[21] Guldhe A, Kumari S, Ramanna L, Ramsundar P, Singh P, Rawat I, Bux F. Review prospects, recent advancements and challenges of different wastewater streams for microalgal cultivation. Journal of Environmental Management. 2017;**203**:299-315. DOI: 10.1016/j.jenvman.2017.08.012

[22] Arias DM, Solé-Bundó M, Garfí M, Ferrer I, García J, Uggetti E. Integrating microalgae tertiary treatment into activated sludge systems for energy and nutrients recovery from wastewater. Bioresource Technology. 2018;**247**:513-519. DOI: 10.1016/j.biortech.2017.09.123

[23] Olaizola M. Commercial development of microalgal biotechnology: From the test tube to the marketplace. Biomolecular Engineering. 2003;**20**(4-6):459-466. DOI: 10.1016/S1389 0344(03)00076-5

[24] Sathasivam R, Radhakrishnan R, Hashem A, Abd_Allah EF. Microalgae metabolites: A rich source for food and medicine. Saudi Journal of Biological Sciences. 2017 . In Press. DOI: 10.1016/j.sjbs.2017.11.003

[25] Pulz O, Gross W. Valuable products from biotechnology of microalgae. Applied Microbiology and Biotechnology. 2004;**65**:635-648. DOI: 10.1007/s00253-004-1647-x

[26] Hu J, Nagarajan D, Zhang Q, Chang J-S, Lee D-J. Heterotrophic cultivation of microalgae for pigment production: A review. Biotechnology Advances. 2018;**36**:54-67. DOI: 10.1016/j.biotechadv.2017.09.009

[27] Wang M, Park C. Investigation of anaerobic digestion of *Chlorella* sp. and Micractinium sp. grown in high-nitrogen wastewater and their co-digestion with waste activated sludge. Biomass and Bioenergy. 2015;**80**:30-37. DOI: 10.1016/j.biombioe.2015.04.028

[28] Borja R, Rincón B. Biogas production. In: Reference Module in Life Sciences. Vol. 2017. United Kingdom: Elsevier; 2017. pp. 1-24. DOI: 10.1016/B978-0-12-809633-8.09105-6

[29] Mussgnug JH, Klassen V, Schlüter A, Kruse O. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. Journal of Biotechnology. 2010;**150**:51-56. DOI: 10.1016/j.jbiotec.2010.07.030

[30] Golueke CG, Oswald WJ, Gotaas HB. Anaerobic digestion of algae. Applied Microbiology. 1957;**5**:47-55

[31] Samson R, LeDuy A. Detailed study of anaerobic digestion of -Spirulina maxima algae biomass. Biotechnoly and Bioengergy. 1986;**28**:1014-1023. DOI: 10.1002/bit.260280712

[32] Mata-Alvarez J, Dosta J, Romero-Güiza MS, Fonoll X, Peces M, Astals SA. A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renewable and Sustainable Energy Reviews. 2014;**36**:412-427. DOI: 10.1016/j.rser.2014.04.039

[33] Mata-Alvarez J, Macé S, Llabrés P. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresource Technology. 2000;**74**:3-16. DOI: 10.1016/S0960-8524(00)00023-7

[34] Hartmann H, Ahring BK. Anaerobic digestion of the organic fraction of municipal solid waste: Influence of co-digestion with manure. Water Research. 2005;**39**(8):1543-1552. DOI: 10.1016/j.watres.2005.02.001

[35] Torres A, Fermoso FG, Rincón B, Bartacek J, Borja R, Jeison D. Challenges for cost-effective microalgae anaerobic digestion. In: Chamy R, editor. Biodegradation – Engineering and Technology. Rijeka: Intech; 2013. pp. 139-159. DOI: 10.5772/55975

[36] Fernández-Rodríguez MJ, Rincón B, Fermoso FG, Jimenez AM, Borja R. Assessment of two-phase olive mill solid waste and microalgae co-digestion to improve methane production and process kinetics. Bioresource Technology. 2014;**157**:263-269. DOI: 10.1016/j.biortech.2014.01.096

[37] Fermoso FG, Beltran C, Jiménez A, Fernández-Rodríguez MJ, Rincón B, Borja R. Screening of biomethane production potential from dominant microalgae. Journal of Environmental Science and Health, Part A. 2016;**51**:1062-1067. DOI: 10.1080/10934529.2016.1198627

[38] Rashid N, Park WK, Selvaratman T. Review: Binary culture of microalgae as an integrated approach for enhanced biomass and metabolites productivity, wastewater treatment and bioflocculation. Chemosphere. 2018;**194**:67-75. DOI: 10.1016/j.chemosphere.2017.11.108

[39] Wang L, Min M, Li Y, Chen P, Liu Y, Wang Y, Ruan R. Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. Applied Biochemistry and Biotechnology. 2010;**162**:1174-1186. DOI: 10.1007/s12010-009-8866-7

[40] Osundeko O, Pittman JK. Implications of sludge liquor addition for wastewater-based open pond cultivation of microalgae for biofuel generation and pollutant remediation. Bioresource Technology. 2014;**152**:355-363. DOI: 10.1016/j.biortech.2013.11.035

[41] Lu W, Wang Z, Wang X, Yuan Z. Cultivation of *Chlorella* sp. using raw dairy wastewater for nutrient removal and biodiesel production: Characteristics comparison of indoor bench-scale and outdoor pilot-scale cultures. Bioresource Technology. 2015;**192**:382-388. DOI: 10.1016/jbiortech.2015.05.094

[42] Cabanelas ITD, Arib Z, Chinalia FA, Souza CO, Perales JA, Almeida PF, Druzian JI, Nascimento IA. From waste to energy: Microalgae production in wastewater and glycerol. Applied Energy. 2013;**109**:283-290. DOI: 10.1016/j.apenergy.2013.04.023

[43] Ebrahimian A, Kariminia HR, Vosoughi M. Lipid production in mixotrophic cultivation of *Chlorella vulgaris* in a mixture of primary and secondary municipal wastewater. Renewable Energy. 2014;**71**:502-508. DOI: 10.1016/j.renene.2014.05.031

[44] Ansari FA, Singh P, Guldhe A, Bux F. Microalgal cultivation using aquaculture wastewater: Integrated biomass generation and nutrient remediation. Algal Research. 2017;**21**:169-177. DOI: 10.1016/j.algal.2016.11.015

[45] Ramanna L, Guldhe A, Rawat I, Bux F. The optimization of biomass and lipid yields of *Chlorella sorokiniana* when using wastewater supplemented with different nitrogen sources. Bioresource Technology. 2014;**168**:127-135. DOI: 10.1016/j.biortech.2014.03.064

[46] Lee KY, Mg TW, Li G, An T, Kwan KK, Chan KM, Huang G, Yip HY, Wong PK. Simultaneous nutrient removal, optimised $CO_2$ mitigation and biofuel feedstock production by *Chlorogonium* sp. grown in secondary treated non-sterile saline sewage effluent. Journal of Hazardous Materials. 2015;**297**:241-250. DOI: 10.1016/j.jhazmat.2015.04.075

[47] Ras M, Lardon L, Bruno S, Bernet N, Steyer JP. Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*. Bioresource Technology. 2011;**102**:200-206. DOI: 10.1016/j.biortech.2010.06.146

[48] Ho SH, Huang SW, Chen CY, Hasunuma T, Kondo A, Chang JS. Bioethanol production using carbohydrate-rich microalgae biomass as feedstock. Bioresource Technology. 2013;**135**:191-198. DOI: 10.1016/j.biortech.2012.10.015

[49] Kamaruddin KF, Yaakob Z, Rajkumar R, Takriff MS, Tasrin SM. Bioremediation of palm oil mill effluents (POME) using *Scenedesmus dimorphus* and *Chlorella vulgaris*. Advances. Science Letters. 2013;**19**:2914-2918. DOI: 10.1166/asl.2013.5044

[50] Bich NN, Yaziz MI, Bakti NAK. Combination of *Chlorella vulgaris* and *Eichhrnia crassipes* for wastewater nitrogen removal. Water Research. 1999;**33**:2357-2362. DOI: 10.1016/S0043-1354(98)00439-4

[51] Nordin AA, Kadir BM, Karimi AB. Treatment of rubber effluent with high rate algal pond. Proc. Rubber Research Institute of Malaysia, PL TRS Conference, Kuala Lumpur; 1989

[52] El-Kassas HA, Mohamed LA. Bioremediation of textile waste effluent by *Chlorella vulgaris*. Egyptian Journal of Aquatic Research. 2014;**40**:301-308. DOI: 10.1016/j.ejar.2014.08.003

[53] Acuner E, Dilek FB. Treatment of textile yellow 2G by Chlorella vulgaris. Process Biochemistry. 2004;**39**:623-631. DOI: 10.1016/S0032-9592(03)00138-9

[54] Lim SL, Chu WL, Phang SM. Use of *Chlorella vulgaris* for bioremediation of textile wastewater. Bioresource Technology. 2010;**101**:7314-7322. DOI: 10.1016/j.biortech.2010.04.092

[55] Ji MK, Yun HS, Park YT, Kabra AN, Oh IH, Choi J. Mixotrophic cultivation of a microalga *Scenedesmus obliquus* in municipal wastewater supplemented with food wastewater and flue gas $CO_2$ for biomass production. Journal of Environmental Management. 2015;**159**:115-120. DOI: 10.1016/j.jenvman.2015.05.037

[56] Shanab S, Essa A, Shalaby E. Bioremoval capacity of three heavy metals by some microalgae species. Plant Signaling & Behaviour. 2012;**7**:1-8. DOI: 10.4161/psb.19173

[57] Toledo-Cervantes A, Morales M, Novelo E, Revah S. Carbon dioxide fixation and lipid storage by *Scenedesmus obliquus*. Bioresource Technology. 2013;**130**:652-658. DOI: 10.1016/j.biortech.2012.12.081

[58] Ergene A, Ada K, Tan S, Katircioglu H. Removal of Remazol brilliant blue R dye from aqueous solution by adsorption onto immobilized *Scenedesmus quadricauda*: Equilibrium and kinetic modelling studies. Desalination. 2009;**249**:1308-1314. DOI: 10.1016/j.desal.2009.06.027

[59] Fazal T, Mushtaq A, Rehman F, Khan AU, Rashid N, Farooq W, Rehman MSU, Xu J. Bioremediation of textile wastewater and successive biodiesel production using microalgae. Renewable and Sustainable Energy Reviews. 2018;**82**:3107-3126. DOI: 10.1016/j.reser.2017.10.029

[60] Liu Y, Yildiz I. The effect of salinity concentration on algal biomass production and nutrient removal from municipal wastewater by *Dunaliella salina*. International Journal of Energy Research. 2018;**39**:1-10. DOI: 10.1002./er.3967

[61] Fouilland E, Vasseur C, Leboulanger C, Le Floc'h E, Carré C, Marty B, Steyer JP, Sialve B. Coupling algal biomass production and anaerobic digestion: Production assessment of some native temperate and tropical microalgae. Biomass and Bioenergy. 2014;**70**:564-569. DOI: 10.1016/j.biombioe.2014.08.027

[62] Torres EM, Hess D, McNeil BT, Guy T, Quinn JC. Impact of inorganic contaminants on microalgae productivity and bioremediation potential. Ecotoxicology and Environmental Safety. 2017;**139**:367-376. DOI: 10.1016/j.ecoenv.2017.01.034

[63] Dong B, Kimberly NH, Ogden KL, Arnold RG. Cultivation of *Nannochloropsis salina* in municipal wastewater or digester centrate. Ecotoxicology and Environmental Safety. 2014;**103**:45-53. DOI: 10.1016/j.ecoenv.2014.02.001

[64] Yeesang C, Cheirslip B. Low-cost production of green microalga *Botryococcus braunii* biomass with high lipid content through mixotrophic and photoautotrophic cultivation. Applied Biochemistry and Biotechnology. 2014;**174**:116-129. DOI: 10.1007/s12010-014-1041-9

[65] Liu J, Ge Y, Cheng H, Wu L, Tian G. Aerated swine wastewater: A promising alternative medium for *Botryococcus braunii* cultivation in open system. Bioresource Technology. 2013;**139**:190-194. DOI: 10.1016/j.biortech.2013.04.036

[66] Yonezawa N, Matsuura H, Shiho M, Kaya K, Watanabe MM. Effects of soybean curd wastewater on the growth and hydrocarbon production of *Botryococcus braunii* strain BOT-22. Bioresource Technology. 2012;**109**:304-307. DOI: 10.1016/j.biortech.2011.07.090

[67] Piligaev AV, Sorokina KN, Shashkov MV, Parmon VN. Screening and comparative metabolic profiling of high lipid content microalgae strains for application in wastewater treatment. Bioresource Technology. 2018;**250**:538-547. DOI: 10.1016/j.biortech.2017.11.063

[68] Santana H, Cerijo CR, Teles VC, Nascimento RC, Fernandes MS, Brunale P, Campanha RC, Soares IP, Silva FCP, Sabaini PS, Siqueira FG, Brasil BSAF. Microalgae cultivation in sugarcane vinasse: Selection, growth and biochemical characterization. Bioresource Technology. 2017;**228**:133-140. DOI: 10.1016/j.biortech.2016.12.075

[69] Suganya T, Varman M, Masjuki HH, Renganathan S. Microalgae as a potential source for commercial applications along with biofuels production: A biorefinery approach. Renewable and Sustainable Energy Reviews. 2016;**55**:909-941. DOI: 10.1016/j.rser.2015.11.026

[70] Phang SM, Miah MS, Yeoh BG, Hashim MA. Spirulina cultivation in sago starch factory wastewater. Journal of Applied Phycology. 2000;**12**:395-400. DOI: 10.1023/A:1008157731731

[71] Zainal A, Yaakob MS, Takriff MS, Rajkumar R, Jaharah AG. Phytoremediation in anaerobically digested palm oil mill effluent using cyanobacterium, *Spirulina platensis*. Journal of Biobased Materials and Bioenergy. 2012;**6**:1-6. DOI: 10.1166/jbmb.2012.1306

[72] Cardoso NF, Lima EC, Royer B, Bach MV, Dotto GL, Pinto LAA. Comparison of *Spirulina platensis* microalgae and commercial activated carbon as adsorbents for the removal of reactive red 120 dye from aqueous effluents. Journal of Hazardous Materials. 2012;**241-242**:146-153. DOI: 10.1016/j.jhazmat.2012.09.026

[73] Cheng Q, Deng F, Li H, Qin Z, Wang M, Li J. Nutrient removal from the secondary effluents of municipal domestic wastewater by *Oscillatoria tenuis* and subsequent co-digestion with pig manure. Environmental Technology. 2017;**12**:1-8. DOI: 10.1080/09593330.2017.1375020

[74] Qin L, Wang Z, Sun Y, Shu Q, Feng P, Zhu L, Xu J, Yuan Z. Microalgae consortia cultivation in dairy wastewater to improve the potential of nutrient removal and biodiesel feedstock production. Environmental Science and Pollution Research. 2016, 2016;**23**:8379-8387. DOI: 10.1007/s11356-015-6004-3

[75] De Schamphelaire L, Verstraete W. Revival of the biological sunlight-to-biogas energy conversion system. Biotechnology Bioengenieering. 2009;**103**(2):296-304. DOI: 10.1002/bit.22257

[76] González-González LM, Correa DF, Ryan S, Jensen PD, Pratt S, Schenk PM. Integrated biodiesel and biogas production from microalgae: Towards a sustainable closed loop through nutrient recycling. Renewable and Sustainable Energy Reviews. 2018;**82**:1137-1148. DOI: 10.1016/j.rser.2017.09.091

[77] Ajeej A, Thanikal JV, Narayanan CM, Senthil Kumar R. An overview of bio augmentation of methane by anaerobic co-digestion of municipal sludge along with microalgae and waste paper. Renewable and Sustainable Energy Reviews. 2015;**50**:270-276. DOI: 10.1016/j.rser.2015.04.121

[78] Geider R, La Roche J. Redfield revisited: Variability of C:N:P in marine microalgae and its biochemical basis. European Journal of Phycology. 2002;**37**:1-17. DOI: 10.1017/S0967026201003456

[79] Heerenklage J, Maxfield T, Zapf A, Adwiraah H, Koerner I. Environmental Sanitary Engineering Centre (CISA): Venice, Italy, 2010Anaerobic Digestion of Microalgae-Possibilities and Limits. Venice 2010, Third International Symposium of Energy from Biomass and Waste, Venice, Italy, CISA. Italy: Environmental Sanitary Engineering Center; 2010

[80] Ehimen EA, Connaugthon S, Sun Z, Carrigton GC. Energy recovery from lipids extracted, transesterified and glycerol co-digested microalgae biomass. Global Change Biology. Bioenergy. 2009;**1**:371-381. DOI: 10.1111/j.1757-1707.2009.01029.x

[81] Wang M, Sahu AK, Rusten B, Park C. Anaerobic co-digestion of microalgae *Chlorella* sp. and waste activated sludge. Bioresource Technology. 2013;**142**:585-590. DOI: 10.1016/j.biortech.2013.05.096

[82] Li R, Duan N, Zhang Y, Liu Z, Li B, Zhang D, Dong T. Anaerobic co-digestion of chicken manure and microalgae *Chlorella* sp.: Methane potential, microbial diversity and synergistic impact evaluation. Waste Management. 2017;**68**:120-127. DOI: 10.1016/j.wasman.2017.06.028

[83] Rétfalvi T, Szabó P, Hájos A-T, Albert L, Kovács A, Milics G, Neményi M, Lakatos E, Ördög V. Effect of co-substrate feeding on methane yield of anaerobic digestion of *Chlorella vulgaris*. Journal of Applied Phycology. 2016;**28**:2741-2752. DOI: 10.1007/s10811-016-0796-5

[84] Beltrán C, Jeison D, Fermoso FG, Borja R. Batch anaerobic co-digestion of waste activated sludge and microalgae (*Chlorella sorokininana*) at mesophilic temperature. Journal of Environmental Science and Health Part A. 2016;**51**(10):847-850. DOI: 10.1080/10934529.2016.1181456

[85] Rusten B, Sahu AK. Microalgae growth from nutrient recovery from sludge liquor and production of renewable bioenergy. Water Science and Technology. 2011;**64**:1195-1201. DOI: 10.2166/wst.2011.722

[86] Mahdy A, Fotidiis IA, Mancini E, Ballesteros M, González-Fernández C, Angelidaki I. Ammonia tolerant inocula provide a good base for anaerobic digestion of microalgae in third generation biogas process. Bioresource Technology. 2017;**225**:272-278. DOI: 10.1016/j.biortech.2016.11.086

[87] Ramos-Suárez JL, Carreras N. Use of microalgae for biogas production. Chemical Engineering Journal. 2014;**242**:86-95. DOI: 10.1016/j.cej.2013.12.053

[88] Astals S, Musenze R, Bai X, Tannock S, Tait S, Pratt S, Jensen PD. Anaerobic co-digestion of pig manure and algae: Impact of intracellular algal products recovery on co-digestion performance. Bioresource Technology. 2015;**181**:97-104. DOI: 10.1016/j.biortech.2015.01.039

[89] Tukaj Z, Bohdanowicz J. Sensitivity to fuel diesel oil and cell wall structure of some Scenedesmus (*Chlorococcales strains*). Acta Societatis Botanicorum Poloniae. 1995;**64**:139-147. DOI: 10.5586/asbp.1995.018

[90] Schwede S, Kowalczyk A, Gerber M, Span R. Anaerobic co-digestion of the marine microalga *Nannochloropsis salina* with energy crops. Bioresource Technology. 2013;**148**:428-435. DOI: 10.1016/jbiortech.2013.08.157

[91] Oleskowicz-Popiel P, Lisiecki P, BoHolm-Nielsen J, Thomsen AB, Thomsen MH. Ethanol production from maize silage as lignocellulosic biomass in anaerobically digested and wet-oxidized manure. Bioresource Technology. 2008;**99**(13):5327-5334. DOI: 10.1016/j.biortech.2007.11.029

[92] Neumann P, Torres A, Fermoso FG, Borja R, Jeison D. Anaerobic co-digestion of lipid-spent microalgae with waste activated sludge and glycerol in batch mode. International Biodeterioration and Biodegradation. 2015;**100**:85-88. DOI: 10.1016/j.ibiod.2015.01.020

[93] Caporgno MP, Trobajo R, Caiola N, Ibáñez C, Fabregat A, Bengoa C. Biogas production from sewage sludge and microalgae co-digestion under mesophilic and thermophilic conditions. Renewable Energy. 2015;**75**:374-380. DOI: 10.1016/j.renene.2014.10.019

[94] Tokuşoglu Ö, üUnal MK. Biomass nutrient profiles of three microalgae: *Spirulina platensis*, *Chlorella vulgaris*, and *Isochrisis galbana*. Journal of Food Science. 2003;**68**(4):1144-1148. DOI: 10.1111/j.1365-2621.2003.tb09615.x

[95] Herrmann C, Kalita N, Wall D, Xia A, Murphy JD. Optimised biogas production from microalgae through co-digestion with carbon-rich co-substrates. Bioresource Technology. 2016;**214**:328-337. DOI: 10.1016/j.biortech.2016.04.119

[96] Li Y, Park SY, Zhu J. Solid-state anaerobic digestion for methane production from organic waste. Renewable and Sustainable Energy Reviews. 2011;**15**:821-826. DOI: 10.1016/j.rser.2010.07.042

[97] Varol A, Ugurlu A. Biogas production from microalgae (*Spirulina platensis*) in a two stage anaerobic system. Waste and Biomass Valorization. 2016;**7**:193-200. DOI: 10.1007/s12649-015-9442-8

[98] Zhen G, Lu X, Kobayashi T, Kumar G, Xu K. Anaerobic co-digestion on improving methane production from mixed microalgae (*Scenedesmus sp*., *Chlorella sp*.) and food waste: Kinetic modeling and synergistic evaluation. The Chemical Engineering Journal. 2016;**299**:332-341. DOI: 10.1016/j.cej.2016.04.118

[99] Solé-Bundó M, Eskicioglu C, Garfí M, Carrère H, Ferrer I. Anaerobic co-digestion of microalgal biomass and wheat straw with and without thermos-alkaline pretreatment. Bioresource Technology. 2017a;**237**:89-98. DOI: 10.1016/j.biortech.2017.03.151

[100] Solé-Bundó M, Cucina M, Folch M, Tàpias J, Gigliotti G, Garfi M, Ferrer I. Assessing the agricultural reuse of the digestate from microalgae anaerobic digestión and co-digestion with sewage sludge. Science of the Total Environment. 2017;**586**:1-9. DOI: 10.1016/j.scitotenv. 201.02.006

[101] Teglia C, Tremier A, Martel JL. Characterization of solid digestates: Part 2, assessment of the quality and suitability for composting of six digested products. Waste and Biomass Valorization. 2011;**2**:113-126. DOI: 10.1007/s12649-010-9059-x

[102] Bjornsson WJ, Nicol RW, Dickinson KE, McGinn PJ. Anaerobic digestates are useful nutrient sources for microalgae cultivation: Functional coupling of energy and biomass production. Journal of Applied Phycology. 2013;**25**:1523-1528. DOI: 10.1007/s10811-012-9968-0

[103] De la Noüe J, Bassères A. Biotreatment of anaerobically digested swine manure with microalgae. Biological Wastes. 1989;**102**:207-214. DOI: 10.1016/0269-7483(89)90100-6

[104] Abeliovich A. Factors limiting algal growth in high rate oxidation ponds. Algae Biomass. 1980:205-215

[105] Blier R, Laliberté G, de la Noüe J. Tertiaary treatment of cheese factory anaerobic effluent with *Phormidium bohneri* and *Micractinium pusillum*. Bioresource Technology. 1995;**52**:151-155. DOI: 10.1016/0960-8524(95)00014-6

[106] Olguin EJ, Hernández B, Araus A, Camacho R, González R, Ramirez ME, Galicia S, Mercado G. Simultaneous high-biomass protein production and nutrient removal using *Spirulina maxima* in sea water supplemented with anaerobic effluents. World Journal of Microbiology and Biotechnology. 1994;**10**:576-578. DOI: 10.1007/BF00367671

[107] Koutra W, Grammatikopoulos G, Kornaros M. Microalgal post-treatment of anaerobically digested agro-industrial wastes for nutrient removal and lipids production. Bioresource Technology. 2017;**224**:473-480. DOI: 10.1016/j.biortech.2016.11.022