

Review

# Digestate as Sustainable Nutrient Source for Microalgae—Challenges and Prospects

Lisa Bauer <sup>1</sup>, Karolína Ranglová <sup>2,3</sup>, Jiří Masojídek <sup>2,4</sup> , Bernhard Drosig <sup>1,5</sup> and Katharina Meixner <sup>1,5,\*</sup> 

<sup>1</sup> Tulln Research Site, BEST—Bioenergy and Sustainable Technologies GmbH, Konrad Lorenz Straße 20, 3430 Tulln, Austria; lisa.bauer@best-research.eu (L.B.); bernhard.drosig@best-research.eu (B.D.)

<sup>2</sup> Laboratory of Algal Biotechnology, Centre Algatech, Institute of Microbiology of the Czech Academy of Sciences, Novohradská 237, 379 81 Třeboň, Czech Republic; ranglova@alga.cz (K.R.); masojidekj@seznam.cz (J.M.)

<sup>3</sup> Faculty of Agriculture, University of South Bohemia, 370 05 České Budějovice, Czech Republic

<sup>4</sup> Faculty of Science, University of South Bohemia, 370 05 České Budějovice, Czech Republic

<sup>5</sup> Institute for Environmental Biotechnology, Department IFA-Tulln, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz Straße 20, 3430 Tulln, Austria

\* Correspondence: katharina.meixner@best-research.eu

**Featured Application:** This review provides insight into the origin of digestate and how it can be processed to meet requirements for microalgae cultivation as well as challenges that occur in cultivation, in downstream processing, and in terms of products when using this nutrient source.

**Abstract:** The interest in microalgae products has been increasing, and therefore the cultivation industry is growing steadily. To reduce the environmental impact and production costs arising from nutrients, research needs to find alternatives to the currently used artificial nutrients. Microalgae cultivation in anaerobic effluents (more specifically, digestate) represents a promising strategy for increasing sustainability and obtaining valuable products. However, digestate must be processed prior to its use as nutrient source. Depending on its composition, different methods are suitable for removing solids (e.g., centrifugation) and adjusting nutrient concentrations and ratios (e.g., dilution, ammonia stripping). Moreover, the resulting cultivation medium must be light-permeable. Various studies show that growth rates comparable to those in artificial media can be achieved when proper digestate treatment is used. The necessary steps for obtaining a suitable cultivation medium also depend on the microalgae species to be cultivated. Concerning the application of the biomass, legal aspects and impurities originating from digestate must be considered. Furthermore, microalgae species and their application fields are essential criteria when selecting downstream processing methods (harvest, disintegration, dehydration, product purification). Microalgae grown on digestate can be used to produce various products (e.g., bioenergy, animal feed, bioplastics, and biofertilizers). This review gives insight into the origin and composition of digestate, processing options to meet requirements for microalgae cultivation and challenges regarding downstream processing and products.

**Keywords:** sustainable nutrients; digestate treatment; nutrient requirements; microalgae cultivation; downstream processing; biorefinery



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## 1. Introduction

The term microalgae covers a great variety of phototrophic microorganisms of various cell shapes and sizes in diverse habitats and thus with distinct requirements concerning cultivation conditions and nutrients [1]. Due to their similar appearance and applications, not only eukaryotic but also prokaryotic cells (cyanobacteria) are often encompassed when the term is used, as done in this review [2]. Recently, microalgae and products thereof have

been gaining more and more attention, which is reflected in the growing amount of produced biomass. According to Acién et al., the worldwide annual production of microalgae in 2019 was 30,000 tons [3]. In Europe, around 60 companies are producing microalgae, mainly in photobioreactors and only a minor share in open ponds [4]. Worldwide, the most prominent microalgae that are cultivated commercially are *Chlorella*, *Arthrospira* (since 2018 also known as *Limnospira* [5] and earlier as *Spirulina*), *Haematococcus*, and *Dunaliella* [4,6].

Both *Chlorella* and *Arthrospira* are rich in protein, polysaccharides, lipids, vitamins, and carotenoids [7,8]. Therefore, they are used as nutritional substitute for a healthy diet. *Dunaliella salina* and *Haematococcus pluvialis* are primarily cultivated for the production of  $\beta$ -carotene and astaxanthin, respectively, which they accumulate under stress conditions (e.g., elevated salt concentrations, nutrient limitation) [9,10]. Both products are used as supplements in diets for humans and animals (e.g., aquaculture) [9,10]. Microalgae can also be used to produce bioenergy. Under stress conditions (e.g., nutrient limitation), the lipid content of *Chlorella* strains can become as high as 50%, which also makes it a potential candidate for the production of biofuel [7]. Recently, new energy-concepts using microalgae have been introduced. After extraction of lipids from microalgal biomass to produce biodiesel, the residual biomass can be fermented in two steps to produce hydrogen and methane, so-called bio-hythane [11]. Moreover, the application of microalgae in microbial fuel cells, producing electricity, is being explored [12].

During microalgae cultivation, nutrients have to be provided besides water, carbon dioxide (CO<sub>2</sub>) and light. Currently, mainly artificial and limited nutrients are used, reducing the sustainability of the process. Moreover, the cultivation costs of EUR 10 kg<sup>-1</sup>, a considerable part of which results from nutrient costs, are high [13]. Thus, the usage of alternative nutrient sources is an important step towards a more ecological and economical microalgae cultivation. By the utilization of waste streams as a nutrient source, the production costs can be reduced to under EUR 5 kg<sup>-1</sup> [14,15]. It provides a reasonable solution, especially for low-value products, e.g., for bioenergy. Anaerobic effluents are a possible alternative nutrient source [16]. Anaerobic effluents (more specifically digestate) are, besides biogas, one of the two products of the anaerobic digestion of biodegradable feedstocks [17]. However, these effluents also contain undesired substances such as particles [17] and have non-transparent and often dark coloration [18], making their utilization in this context not as straightforward.

This review focuses on the arising challenges when using anaerobic effluents in microalgae cultivation and demonstrates possible solutions. In this study, variables that might influence the usage of digestate as nutrient source for microalgae cultivation are revealed. Furthermore, this study points out the different origins and compositions of anaerobic effluents as well as suitable options for removing undesired substances. Moreover, it will be shown how the inculcation of digestate into the production process affects the cultivation and downstream processing and possible applications of the products will be presented.

## 2. Cultivation Conditions

Favorable cultivation conditions are diverse and strongly depend on the microalgae species or strain, as well as on the desired product. Important factors are temperature, pH, salinity, light intensity and spectrum, and nutrients. Typical cultivation variables for *Chlorella*, *Arthrospira*, *Dunaliella*, and *Haematococcus* are listed in Table 1. The variables such as the pH value, salinity, and nutrients directly depend on the nutrient composition of the cultivation medium. Additionally, the light energy that is available for photosynthesis is dependent on the color and transparency of the cultivation medium. When using digestate as nutrient source instead of mineral medium, these variables should especially be considered. Therefore, their significance will be elaborated in this section.

**Table 1.** A coarse overview over typical cultivation variables for commercially popular genera such as *Chlorella*, *Arthrospira*, *Dunaliella*, and *Haematococcus*.

	Unit	<i>Chlorella</i>	<i>Arthrospira</i>	<i>Dunaliella</i>	<i>Haematococcus</i>
Temperature	[°C]	25–30 [19,20]	30–38 [21]	30–40 [19]	18–22 [19]
pH	[-]	6.5–7.5 [19]	9–10 [21]	9–11 [22]	7 [23]
Max. salinity	[% (w/v) NaCl]	1 [19]	<3 [19]	20–35 [19,24]	1 [19]
Typical cultivation medium	[-]	BBM <sup>b</sup> [19,25]/ BG-11 <sup>c</sup> [26]	Zarrouk [19,21]	Modified Johnson [19,22]	BBM <sup>b</sup> [10,19]
N <sup>a</sup>	[mM]	2.94/17.63 [6]	29.40 [6]	9.89 [27]	2.94 [6]
P <sup>a</sup>	[mM]	1.72/0.23 [6]	2.87 [6]	0.26 [27]	1.72 [6]
K <sup>a</sup>	[mM]	2.15/0.46 [6]	17.2 [6]	12.83 [27]	2.15 [6]
N/P ratio <sup>a</sup>	[-]	1.71/76.78 [6]	10.24 [6]	38.46 [27]	1.71 [6]

<sup>a</sup> Concentration/ratio in the given typical cultivation medium, <sup>b</sup> BBM—Bold's Basal Medium [25], <sup>c</sup> BG-11—Blue Green-11 [26].

### 2.1. Temperature, pH, and Salinity

Temperature, pH, and salinity preferences vary among species and are usually reflected by their natural habitat. As shown in Table 1, commercially produced microalgae are cultivated at warm conditions and either neutral or slightly alkaline pH values. Additionally, it must be considered that temperature, pH, and salinity determine the solubility and volatility, and therefore the availability of nutrients and other substances present in the cultivation medium [17,28].

The pH of a nutrient solution can be adjusted by the addition of acids, bases, or CO<sub>2</sub>. It should be controlled during the cultivation because it is important for photosynthesis. Depending on the pH value, dissolved CO<sub>2</sub> is available in water in different forms [29]. Most microalgae prefer taking up CO<sub>2</sub> or HCO<sub>3</sub><sup>-</sup>; the latter is available at a pH value between 6.5 and 10 [29].

The highest tolerated salinity strongly differs among species (see Table 1). Extreme salt concentrations lead to various reactions in the microalgal cell, as the cell attempts to survive and adjust to osmotic pressure to establish a new steady state for growth [30]. Reactions include the restoration of turgor pressure, regulation of ion uptake and release, and accumulation of osmo-protecting solutes [31]. Under salt stress, photosynthetic activity is reduced due to an increase in respiratory activity and the inhibition of photosystem II [30]. Excess energy resulting from reduced growth can be stored in form of carbohydrates or lipids [32].

### 2.2. Light

During photosynthesis, light energy is used to convert inorganic carbon into organic compounds. Light is either provided naturally by the sun or artificial sources, depending on the location and purpose. If the intensity is too low, growth is limited, but if it exceeds a certain threshold, photo-inhibition occurs due to excess energy [33]. The optimum light intensity varies, but usually lies between 60 and 2000 μmol m<sup>-2</sup> s<sup>-1</sup> [34]. When a light beam travels through the liquid culture, there is a gradient from high light intensity at the outer layers to low intensity at the center. It is attenuated by the cultivation medium, which can especially be a problem if it is not transparent but of a dark color. This has to be kept in mind when digestate is used as nutrient source. Additionally, microalgal cells absorb light as they need it for oxygenic photosynthesis. Thus, special care has to be taken during the photobioreactor design (i.e., layer thickness, mixing) to make sure every cell in the culture is exposed to suitable light intensities [35].

Furthermore, when choosing artificial light, not only the light intensity but also the spectral composition is of great importance [33]. For photosynthesis, the wavelength range from 400 to 700 nm is relevant [34]. The optimum light spectrum varies, as the pigment composition differs between species [34]. The pigment composition also depends on availability of nutrients in the cultivation medium [36,37]. In microalgae cells, pigments are responsible for capturing the light and directing it through the light harvesting complex

to chlorophyll *a*. Each pigment can only absorb light of certain spectra. Nevertheless, chlorophyll, which absorbs light mainly in the blue (420–470 nm) and red (600–700 nm) spectral range, is dominant in most species [34,38]. Sources that emit light primarily in this range should be chosen in order to use the light energy efficiently in a production process. When using cultivation medium of darker color, the light will be absorbed to a certain degree [18]. This makes choosing the right light source and intensity vital.

### 2.3. Carbon Sources

Depending on the microalgae strain, the cultivation can be performed photoautotrophically, heterotrophically, or mixotrophically [39]. Photoautotrophic cultivation has widely been reported [15,40,41]. For this mode of growth, the cells require an inorganic source of carbon (e.g., CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>) [39]. During mixotrophic cultivation, both organic and inorganic carbon sources are used in presence of light [42]. This cultivation mode shows less photo-limitation than photoautotrophic cultivation and higher biomass productivity as well as nitrogen and phosphorus uptake efficiency [39,43]. When cultivating microalgae heterotrophically, only organic carbon is used. This cultivation mode is also independent of light, and therefore biomass productivities and concentrations are higher than under photoautotrophic conditions [42]. However, not all microalgae strains are able to metabolize organic carbon [42]. Its presence in the cultivation medium leads to a higher possibility of microbial contaminations (e.g., bacteria) [7,39,42]. This issue is discussed in Section 5.3.

### 2.4. Nutrient Requirements

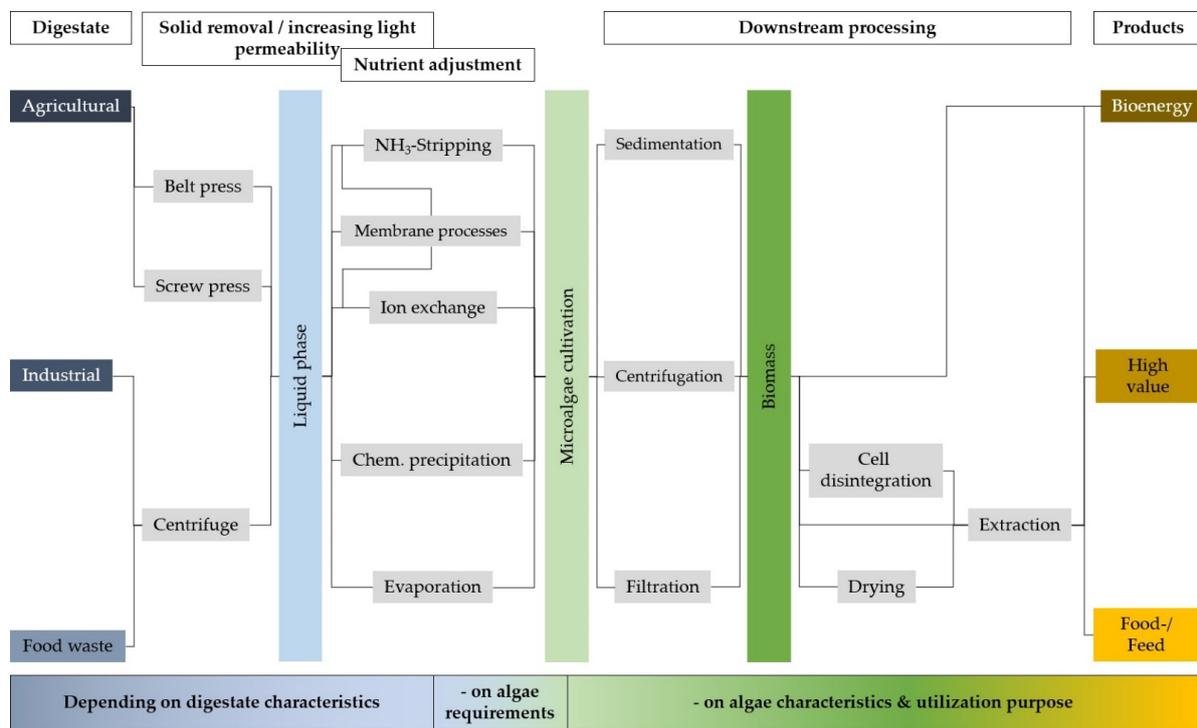
Microalgae are diverse and so are their biomass compositions and nutrient requirements. A successful commercial microalgae production requires that the most important nutrients are provided in sufficient amounts and at more or less balanced ratios. Otherwise, growth or doubling times will be reduced due to the deficiency in one element (Liebig's law of the minimum) [29]. Carbon, hydrogen, and oxygen, which make up 65–85% of the cell dry weight, are fixed through photosynthesis [6]. Required elements must be provided by the cultivation medium. The biomass composition varies among species, but the average composition of planktonic microalgae was found to be 7.6% N, 2.5% Na, 2.3% K, 1.4% S, and 1.1% P [6]. This can be a general guideline for the nutrient composition of the cultivation medium [6]. However, microalgae are capable of adapting their nutrient uptake to availabilities, which can also be exploited in biotechnology if secondary metabolites are desired [29]. Nitrogen starvation can trigger the production of  $\beta$ -carotene in *Dunaliella* or the accumulation of storage lipids as well as polysaccharides in various microalgae [7,42]. A limitation of nitrogen or the combination of nitrogen and phosphorus depletion leads to astaxanthin production in *Haematococcus* and polyhydroxybutyrate (PHB) accumulation in many cyanobacteria [10,44].

Various cultivation media have been developed for different species or strains and their specific applications. Nevertheless, media are usually not optimized for the use in mass cultivation but slightly modified laboratory media are often used [45]. Most have excess nutrients, and thus growth is not at its optimum and their use is neither economical nor sustainable [45]. Furthermore, mainly artificial and limited nutrient resources are currently used. To reduce the ecological impact of microalgae cultivation and increase its sustainability, the utilization of alternative nutrient sources such as anaerobic effluents are mandatory in the long run. This would also reduce costs and dependencies from producing countries and strengthen regional nutrient supply.

### 2.5. Digestate as Nutrient Source

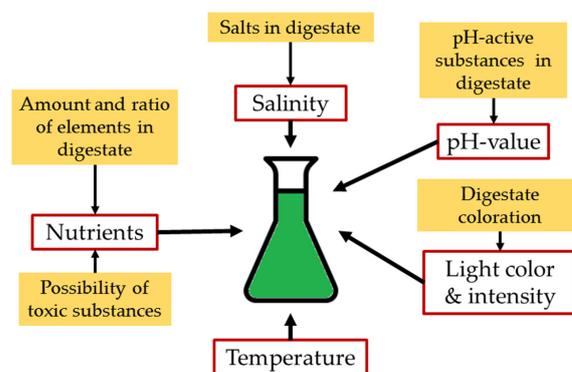
Digestate occurs in large quantities as residue of biogas production and contains valuable nutrients. Therefore, and as proven by various publications, it is considered a potential nutrient source for microalgae cultivation (Section 5). When using digestate as nutrient source, different aspects along the whole process chain (raw digestate, digestate treatment,

microalgae cultivation, downstream processing, and products) must be considered. An overview of the steps is given in Figure 1.



**Figure 1.** Overview of possible process steps for obtaining microalgae products by using digestate as nutrient source. Solids separation during digestate processing and microalgae harvesting can be enhanced by using precipitating or flocculating agents.

Most of the variables described above are influenced when digestate is used as nutrient source instead of mineral medium [17,46–48]. The composition of anaerobic effluents is directly connected to the feedstock used for the anaerobic digestion, which is described below in more detail (Sections 3 and 4). The components have a direct effect on the available nutrients, pH value, and salinity of the cultivation medium (Figure 2). In turn, the cultivation temperature and pH value determine the solubility of nutrients as well as potentially harmful substances present in the digestate. Additionally, solids or other components lead to a dark coloration of the cultivation medium, which means that light of certain wavelengths is absorbed. Thus, the light energy available for photosynthesis is decreased. To conclude, complex interdependencies of variables determine the organism’s environment and its growth performance in every cultivation.



**Figure 2.** Overview of important variables for microalgae cultivation and the possible influences of digestate as nutrient source.

### 3. Origin of Digestate

Digestate or anaerobic effluents arise when carbon of organic material is converted to biogas under anaerobic conditions, mainly consisting of methane (CH<sub>4</sub>—energetic component) and CO<sub>2</sub>. Most macro- (N, P, K, S, Mg, Ca) and micronutrients (B, Cl, Cu, Fe, Mn, Mo, Ni, Zn) are concentrated in digestate [46]. However, digestate also contains solids (suspended matter) and colorants. The raw material affects the TS (total solids—fiber, particles, soluble solids) and nutrient content (N, P, K) of the digestate [17,46–48]. An overview of the nutrient composition of different digestates in terms of their origin is summarized in Table 2. On the basis of the used raw material, biogas plants can be categorized into agricultural, industrial, and waste plants [17,46–48]. Agricultural plants use agricultural residues as well as renewable materials, industrial plants use various organic residues ranging from whey to slaughterhouse residues and wastewater, whereas different kinds of food wastes are used in waste plants [17]. Agricultural residues and renewable raw materials such as maize silage result in high dry weight contents (6–24%), while food waste and liquid industrial residues usually result in rather low TS concentrations [17]. High protein contents in the raw material such as in slaughterhouse wastes lead to high NH<sub>4</sub>-N concentrations [17]. Colorants, high solid and NH<sub>4</sub>-N concentrations may cause severe problems in microalgae cultivation since light is absorbed over the whole visible spectrum [18], and too high NH<sub>4</sub>-N concentrations may have toxic effects on microalgae [49]. Digestate also contains inorganic (CaCO<sub>3</sub>) and organic carbon (volatile fatty acids), whose concentrations depend on pH value and on the degree of digestion [17].

During the anaerobic digestion process, trace elements (microelements) are often added, especially in the case of mono-digestion [17]. Furthermore, additives such as iron chloride are sometimes used to remove sulfur compounds. These additives might influence microalgal growth if present in the digestate.

**Table 2.** Origin and composition of anaerobic effluents.

Origin	Raw Material	pH [-]	TS <sup>a</sup> [%]	VS <sup>b</sup> [%]	NH <sub>4</sub> -N <sup>c</sup> [g kg <sup>-1</sup> ]	TKN <sup>d</sup> [g kg <sup>-1</sup> ]	P <sup>e</sup> [g kg <sup>-1</sup> ]	References
Agricultural residues/renewable materials	n.d. <sup>f</sup>	7.5–8.4	6.41–24	4.42–18.5	0.03–4.1	0.09–5.04	0.46–5.76	[46]*
	Co-digestion manure + crops and/or industrial waste	5.6–8.3	1.5–24	0.93–18.5	0.01–1.63	0.02–12.1	0.002–2.4	[46]*
	Corn silage, manure, agricultural residues	7.7–8.1	6.1–8.3	4.4–6.3	4.9–6.1	7.6–9.6	n.d. <sup>f</sup>	[17]
	Crop digestion with manure	7.7–8.0	6.5–8.6	4.8–6.4	2.3–4.2	4.3–6.1	n.d. <sup>f</sup>	[17]
	Crop digestion	7.4–7.9	6.2–8.6	4.8–6.2	1.5–2.5	3.6–5.2	n.d. <sup>f</sup>	[17]
	Crop digestion	7.2–7.9	7.8–9.0	5.7–6.7	1.3–3.6	4.6–6.3	n.d. <sup>f</sup>	[17]
	Corn and grass silage	7.6–8.0	6.6–9.3	4.8–6.9	1.3–2.4	3.6–4.9	n.d. <sup>f</sup>	[17]
Industrial residues	Manure	7.3–8.6	2.2–9.2	1.49–6.9	0.06–0.93	0.01–0.57	0.007–0.2	[46]*
	Brewers' spent grains	7.3–7.5	5.3–5.8	4.7–5.3	1.9–2.3	2.3–3.1	n.d. <sup>f</sup>	[17]
	Slaughterhouse waste	7.9–8.3	2.2–4.9	1.6–3.9	5.3–7.7	6.4–8.1	n.d. <sup>f</sup>	[17]
	Thin stillage—bioethanol by-product	7.7–8.1	1.7–2.8	0.9–1.6	2.2–2.8	3.0–4.3	n.d. <sup>f</sup>	[17]
	n.d. <sup>f</sup>	7.9–8.3	1.4–7.88	0.56–5.78	0.01–0.67	0.01–0.98	0.002–0.1	[46]*
Food waste/residues	Bio waste	7.6–8.1	2.5–4.7	1.4–2.7	1.5–5.6	3.0–6.8	n.d. <sup>f</sup>	[17]
	Bio and food waste, blood	8.0–8.3	3.9–4.1	2.4–2.8	5.1–7.2	6.4–8.1	n.d. <sup>f</sup>	[17]
	Bio and food waste	7.3–7.9	1.6–3.3	1.0–1.7	0.6–1.5	1.4–2.3	n.d. <sup>f</sup>	[17]
	Bio and food waste, blood, food industry residues	7.8–8.2	5.6–8.1	3.0–4.5	3.1–4.1	4.2–6.7	n.d. <sup>f</sup>	[17]
	Manure, slaughterhouse, bio, food, and kitchen waste	8.0–8.3	5.7–7.2	4.1–5.6	6.8–8.6	8.4–10.8	n.d. <sup>f</sup>	[17]
	Kitchen food waste	8.0	5.9	n.d. <sup>f</sup>	4.02	n.d. <sup>f</sup>	0.67	[50]

<sup>a</sup> TS—total solids, <sup>b</sup> VS—volatile solids, <sup>c</sup> NH<sub>4</sub>-N—ammonia nitrogen, <sup>d</sup> TKN—total Kjeldahl nitrogen, <sup>e</sup> P—phosphorous, <sup>f</sup> n.d.—not defined/determined, \* values converted into fresh matter.

### 4. Digestate Processing

In agriculture, digestate is often used as fertilizer on fields because it contains various valuable nutrients [47]. Reducing the water content of digestate is beneficial in order to reduce transportation and storage costs [47]. Further, solids can then be used for fertilizing the fields more easily, while the liquid phase remains as nutrient source for microalgae cultivation. To reduce the digestate volume, so-called partial processing is proposed. In contrast, complete processing aims at refining digestate to pure water, fibers, and solids as

well as to mineral nutrients. Technologies used for partial processing are rather simple and cheap while complex technologies are necessary for complete processing, which differ in their degree of technical maturity, energy consumption, and costs [17,47,48].

In the first step of a partial digestate procession, flocculating or precipitating agents are added to improve solids removal. Subsequently, solids can be directly used to fertilize fields, composted, or dried. Nutrient recovery can be carried out via membrane technologies such as ultra- or nano-filtration with subsequent reverse osmosis and separated into nutrient concentrates and purified processed water [17,46,47]. The nitrogen concentration can be reduced or adjusted in digestate or the liquid fraction via stripping, ion exchange, struvite precipitation [17,47,48], and evaporation [46]; membrane conductors [51] have already successfully been tested at lab scale [17,47,48].

#### 4.1. Solid–Liquid Separation

For solid–liquid separation, various technologies are available, e.g., decanter centrifuges, screw press separators, bow sieves, and sieve belt presses. Decanter centrifuges and screw press separators are most commonly used. Choosing the appropriate technology is based on the characteristics of the digestate. Decanter centrifuges are most suitable for small particle sizes and low TS concentrations [52] and are, for example, used in industrial and municipal wastewater treatment plants [17,47,48].

In the solid fraction, the main shares of the elements phosphorous and carbon remain, while soluble compounds such as nitrogen (TN, NH<sub>4</sub>-N) and potassium (K) stay mainly in the liquid fraction (see Table 3) [17,53]. By coupling various efficient technologies such as centrifuge or screw press with filtration, the separation efficiency could be increased. It can further be enhanced by using flocculating and precipitating agents, e.g., aluminum sulfate (Al<sub>2</sub>(SO<sub>3</sub>)<sub>3</sub>), ferric chloride (FeCl<sub>3</sub>), and ferric sulfate (Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>) [46,47,53,54]. There are various additives on a natural basis such as lime (Ca(OH)<sub>2</sub>), functionalized chitosan, cellulose, gums, mucilage, and modified starch, as well as synthetic additives such as polyacrylamide [46,55,56]. Through the addition of inorganic metal salts, the salt concentration in the liquid phase might also increase.

**Table 3.** Distribution of mass and nutrients after solid–liquid separation [17,47].

	Unit	Liquid	Solids
Mass	[%]	80–90	10–20
TS <sup>a</sup>	[%]	40–50	50–60
VS <sup>b</sup>	[%]	35–45	55–65
Ash	[%]	50–60	40–50
TN <sup>c</sup>	[%]	65–75	25–35
NH <sub>4</sub> -N <sup>d</sup>	[%]	70–80	20–30
P <sup>e</sup>	[%]	35–45	55–65
K <sup>f</sup>	[%]	70–80	20–30
C <sup>g</sup>	[%]	30–40	60–70

<sup>a</sup> TS—total solids, <sup>b</sup> VS—volatile solids, <sup>c</sup> TN—total nitrogen, <sup>d</sup> NH<sub>4</sub>-N—total ammonia nitrogen, <sup>e</sup> P—phosphorous, <sup>f</sup> K—potassium, <sup>g</sup> C—carbon.

By adding precipitating agents (lime and FeCl<sub>3</sub>), the separation efficiencies of suspended solids (from 46% to 75%) and P (from 54% to 95%) were increased and that of N (from 18% to 47%) and K (from 10% to 25%) as well. The increase of N can be explained by higher separation of organic nitrogen rather than by NH<sub>4</sub>-N, which is hardly affected by precipitating agents [54].

#### 4.2. Nutrient Removal and Recovery

Membrane technologies can be used to remove particles, suspended solids, and dissolved salts. Particles and colloids down to 0.1 μm (microfiltration), 0.01 μm (ultrafiltration), and 0.001 μm in diameter (nano filtration, including microorganisms) can be separated

with porous membranes. The application of solution-diffusion membranes (reverse osmosis) can separate dissolved salts such as  $\text{NH}_4$  [17,46]. Using microfiltration, 93% of phosphorous and, with a combination of micro-, ultra-, and nano-filtration, 94% of nitrogen could be recovered [50]. Digestate processing via membranes typically includes micro- or ultrafiltration followed by two- to three-stage reverse osmosis, depending on the permeate quality. Permeate (liquid fraction) of membrane processes can for example be used as process or washing water, while retentate is rich in nutrients. Membrane processes are prone to fouling and are rather expensive and energy consuming [17].

Further options to recover, remove, or adjust nitrogen concentration in liquid digestate include ammonia stripping [17,46,57], membrane conductors [51], evaporation [46,58], and membrane distillation [59], as well as struvite precipitation [17,46,60] and ion exchange [17].

The principle of ammonia stripping is removing  $\text{NH}_3$  from the liquid by sparging it with gas and recovering  $\text{NH}_4\text{-N}$  in sulfuric acid in the form of ammonium sulfate ( $(\text{NH}_4)_2\text{SO}_4$ ). The volatility of ammonia can be enhanced by increasing temperature and pH value. The pH can be increased by degassing and removing  $\text{CO}_2$  or adding alkali. Ammonia stripping is carried out in packed columns but also in stirred tank reactors, which are more robust and less prone to clogging due to particles which results in lower maintenance and cleaning effort [17]. Additionally, there is a certain need for chemicals (pH adjustment, sulfuric acid). To recover heat, the utilization of flue gas instead of air is a viable option [57]. One advantage of  $\text{NH}_3$ -stripping is that a standardized pure nitrogen fertilizer can be recovered; this liquid could be used to increase the nutrient content of other digestate fractions and therefore their marketability [17].

Another option to recover  $\text{NH}_4\text{-N}$  in the form of  $(\text{NH}_4)_2\text{SO}_4$  is by using membrane conductors. These membranes are directly submerged into the substrate, and sulfuric acid is pumped through their core as extraction solution. Their application at 400 L-scale has been promising; however, cleaning and maintenance need to be improved [51].

Additionally, evaporation is an attempt to remove  $\text{NH}_4\text{-N}$  or adjust its concentration in digestate, as through addition of sulfuric acid,  $(\text{NH}_4)_2\text{SO}_4$  is formed and less  $\text{NH}_3$  evaporates [58]. This way, small organic acids can be removed besides  $\text{NH}_4\text{-N}$  [46]. To recover nitrogen and phosphorous simultaneously from liquid digestate, research has successfully tested membrane distillation with fractional condensation of permeate on the downstream side of the membrane [59]. Drawbacks of evaporation and other heat-demanding processes are the high energy requirements, and therefore they are only interesting where excess heat is available [17]. Prior to evaporation, solids, especially fibers, often need to be removed and additionally sulfuric acid is needed to adjust the pH value [17].

Nitrogen and/or phosphorous can also be removed by struvite ( $\text{Mg}(\text{NH}_4)\text{PO}_4 \cdot 6\text{H}_2\text{O}$ ) precipitation, which is induced at elevated pH values in the presence of  $\text{NH}_4$ , magnesium (Mg), and phosphate ( $\text{PO}_4^{3-}$ ). For this purpose, magnesium oxide and phosphate ions need to be added in excess and the pH value is increased by the addition of alkali. Chemical demand is the main disadvantage of struvite precipitation. In contrast, an advantage is that struvite can be used as slow-release fertilizer and that there are several established processes for struvite recovery and products [17,46,60]. In addition, removing solids before the precipitation might be advantageous.

Ion exchange is another option to remove  $\text{NH}_4\text{-N}$ , which is hardly used since the liquid needs to be free of particles [17]. It is operated in batch-mode because the ion exchange resins need to be regenerated, causing also a certain demand for chemicals (NaCl) [17].

In most processes, the amount of nutrients that are removed depends on the processing time or cycles and on the use of chemicals—the longer digestate is processed or the more cycles are applied, the more  $\text{NH}_4\text{-N}$  or phosphorus will be removed.

## 5. Cultivation in Digestate

As described above, digestate is characterized by high amounts of nitrogen (total nitrogen content could reach up to  $12 \text{ g L}^{-1}$ ) and phosphorus (total phosphorus could reach up to  $5.8 \text{ g L}^{-1}$ ) and by a slightly alkaline or neutral pH (see Table 2). Thus, digestate in

general is suitable for the cultivation of many different microalgae strains. Various authors have already described the utilization of digestate for microalgae cultivation [44,50,60–62]. Nevertheless, prior to cultivation at a larger scale, it is mandatory to perform preliminary laboratory experiments in each specific case. This is necessary to find out whether the nutrient source is suitable for microalgal cultivation when obtained directly from the anaerobic process. Pre-treatment of the digestate, using some of the methods described above, might be necessary to provide microalgae with a suitable environment and proper nutrient content. As mentioned in Section 2, different microalgae species have different requirements regarding pH, nutrient composition, and salinity. Furthermore, anaerobic effluents differ in, e.g., solids concentration, nutrient composition, pH, and color, depending on the feedstock and the variables used during anaerobic digestion, as described in Sections 3 and 4. Thus, the necessary steps to obtain a suitable cultivation medium strongly depend on the microalgae species to be cultivated as well as on the properties of the digestate. There is no universal solution that can be applied, but rather a pool of options from which the right combination of methods must be chosen for each specific application. The aim of this section is to give an overview of how digestate can be processed prior to cultivation and how this affects growth performance as shown in various studies.

### 5.1. Removal of Solids and Increasing Light Permeability

One of the first steps in digestate pre-treatment could be the removal of solid particles to obtain a homogenous liquid medium. This can be achieved by centrifugation (either with or without adding precipitating agents) and subsequent ultrafiltration of the liquid fraction, as carried out by Meixner et al. [44] (digestate from thin stillage treatment) and Veronesi et al. [61] (digestate from agro-industrial sources). In both studies, the permeate was then diluted with water, which led to a reduced nutrient load and improved light permeability [44,61]. The microalgae strains *Phaeodactylum tricornerutum* and *Pavlova lutheri* were selected for further cultivation on the obtained medium. Both strains performed well in both inorganic f/2 medium and treated digestate. Almost the same biomass productivity was achieved in both media (in digestate and f/2 medium *Pavlova lutheri* reached 15 and 17 mg L<sup>-1</sup> d<sup>-1</sup> and *Phaeodactylum tricornerutum* reached 25 and 24 mg L<sup>-1</sup> d<sup>-1</sup>, respectively) [61]. *Synechocystis* cf. *salina* achieved biomass concentrations of 1.55 ± 0.33 g L<sup>-1</sup> (0.11 ± 0.02 g L<sup>-1</sup> d<sup>-1</sup>) and 1.26 ± 0.45 g L<sup>-1</sup> (0.09 ± 0.03 g L<sup>-1</sup> d<sup>-1</sup>) in digestate supernatant (diluted 1/3 and 1/5 with water) compared to 1.98 ± 0.37 g L<sup>-1</sup> (0.14 ± 0.02 g L<sup>-1</sup> d<sup>-1</sup>) when cultivated in BG-11 medium [44]. However, the growth rate in ultrafiltration permeate (diluted 1/5) was considerably lower (0.23 d<sup>-1</sup> compared to 0.5 d<sup>-1</sup> in supernatant (diluted 1/5) and 0.86 d<sup>-1</sup> in BG-11), which was assumed to result from low Mg concentrations or low N/P ratios [44]. Fernandes et al. [50] chose a different order for digestate treatment—first dilution (various concentrations), then sedimentation, and finally filtration steps were performed. *Chlorella vulgaris* performed better at low concentrations of processed digestate (2.5%). The growth rates in digestate were scalable and converted excess nutrients to biomass. *C. vulgaris* grew well for 28 days with a maximum growth rate of 0.62 d<sup>-1</sup> and a maximum biomass concentration of 0.86 g L<sup>-1</sup>. After each harvest, the culture was supplemented with new digestate medium. As nutrients were not entirely transformed to biomass by these microalgae, an accumulation of ammonium and heavy metals was observed and presumably linked to a decline in growth rate after 28 days.

Depending on its properties, untreated digestate can be used in some cases. When BG-11 medium was supplemented with unprocessed digestate (from municipal sludge treatment) by 10 and 20% (v/v), the growth rate of the wild type *Synechocystis* sp. PCC 6803 increased significantly. At higher supplementation over 20% (v/v), growth was inhibited, probably due to decreased transparency and the culture becoming photo-limited [62]. As mentioned, growth limitation at higher digestate concentration was observed by Fernandes et al. [50] as well.

It should additionally be considered how the effluent to-be-used was treated in the preceding process. During the process, e.g., in a wastewater treatment plant, an automatic addition of flocculant might cause cell aggregation when the wastewater is used to cultivate microalgae. This might be overcome by centrifugation of the activated sludge without flocculant addition [15].

### 5.2. Adjustment of Nutrients

Besides the removal of solids and obtaining a light-permeable cultivation medium, the nutrient concentration in digestate must also be considered. High nutrient loads can simply be lowered by dilution [44,50,61,62]. However, this will not affect the ratio between elements, which is an important variable to induce microalgae reproduction (see Section 2). Thus, if there is a high load of only specific nutrients, the ratio must be adjusted. If solid–liquid separation techniques are applied, it must be considered that some of the nutrients tend to remain in the liquid fractions (supernatant, permeate) while others might remain in the solid fractions (retentate) (see Section 4). Thus, the ratio might change during solid–liquid separation. These techniques can therefore be used for adjusting the nutrients. Alternatively, the digestate could be supplemented with selected nutrients.

Ammonium nitrogen is the major component of total nitrogen (65–98%) in digestate (see Table 2, [39]). Depending on the concentration of macro-elements, the digestate could be diluted to prevent  $\text{NH}_4\text{-N}$  inhibition during microalgae cultivation. If the ratio between the elements is out of balance, the share of ammonium should be reduced. An  $\text{NH}_3\text{-N}$  concentration of 0.3 mM at pH 6 inhibited the growth of *Scenedesmus obliquus* [49]. However, the utilization of this nitrogen source after setting the proper concentration can result in fast growth of microalgae [39]. The  $\text{NH}_4$  uptake by *Neochloris oleoabundans* was efficient (about 90–95%) [13].

Microalgal growth was also achieved in digestate supernatant resulting from struvite precipitation [60]. The highest growth rates were obtained by *Dictyosphaerium ehrenbergianum* ( $0.144\text{ d}^{-1}$ ), followed by *Chlorella regularis* ( $0.097\text{ d}^{-1}$ ), *Scenedesmus obliquus* ( $0.089\text{ d}^{-1}$ ), *Arthrospira maxima* ( $0.084\text{ d}^{-1}$ ), and *Arthrospira subsalsa* ( $0.069\text{ d}^{-1}$ ). In terms of *D. ehrenbergianum* more specifically, higher biomass ( $161\text{ mg L}^{-1}\text{ d}^{-1}$ ) and lipid productivity ( $55\text{ mg L}^{-1}\text{ d}^{-1}$ ) was achieved compared to mineral medium ( $85\text{ mg biomass L}^{-1}\text{ d}^{-1}$ ,  $20\text{ mg lipids L}^{-1}\text{ d}^{-1}$ ), when the  $\text{NH}_4^+/\text{Mg}^{2+}/\text{PO}_4^{3-}$  ratio was adjusted to 1:1.2:1.2.

Besides nutrients, pH value and salinity are important variables for microalgae cultivation (see Section 2). The pH value of digestate usually reaches from neutral to slightly alkaline (see Table 2). This is similar to the required pH value for some commercial microalgae (see Table 1). However, the pH value can change during digestate treatment (e.g., ammonia stripping, struvite precipitation), as described in Section 4. If the pH value of the treated digestate does not fit the microalgae requirements, it needs to be adjusted. The presence of various digestate components or chemicals added during digestate treatment might cause a high salinity, which might have to be lowered through dilution prior to microalgae cultivation.

### 5.3. Organic Carbon in Digestate

If the digestion of feedstock in the biogas plant is not complete, an organic carbon source in the form of volatile fatty acids is present in digestate [17]. This might lead to mixotrophic growth of microalgae, increasing the risk of contamination by heterotrophic organisms (Section 2.3).

Microalgae species such as *Chlorella vulgaris*, *Arthrospira platensis*, and *Haematococcus pluviialis* can grow mixotrophically, which means that they are able to metabolize organic carbon if present [39]. Mixotrophic cultivation mode can improve biomass productivity and nutrient uptake [39]. Light intensity is less critical than in photoautotrophic mode [39]. This is an advantage, with digestate constituents in particular leading to a dark color of the cultivation medium.

However, the availability of organic carbon might lead to contaminations by heterotrophic organisms [63]. This should especially be considered when microalgae are grown in open systems [64]. Bacteria and other contaminating organisms might compete with microalgae for nutrients and inhibit their growth [39]. As sterilization of the cultivation medium is not an economically feasible option for mass culture [39], other strategies to handle this issue have to be carried out. Cultivating strains with high growth rates or microalgae that grow in selective conditions (e.g., hypersaline water—*Dunaliella*, or high pH value—*Arthrospira*) [64,65] are possible options. It is necessary to follow a strict protocol and to monitor the process in order to keep control of the cultivated organisms [39].

## 6. Downstream Processing

Downstream processing of microalgae cultures means any treatment of the culture after cultivation to dewater biomass and isolate desired products. It includes several alternative operations to treat the microalgae culture: harvesting, disintegration, dewatering, and purification (removal of the components with properties significantly different from the products—adsorption, hydrolysis, extraction, precipitation, chromatography, ultrafiltration, fractionation) (for a review see Molina Grima et al. [49]). A brief description of basic operations is presented in Figure 3.

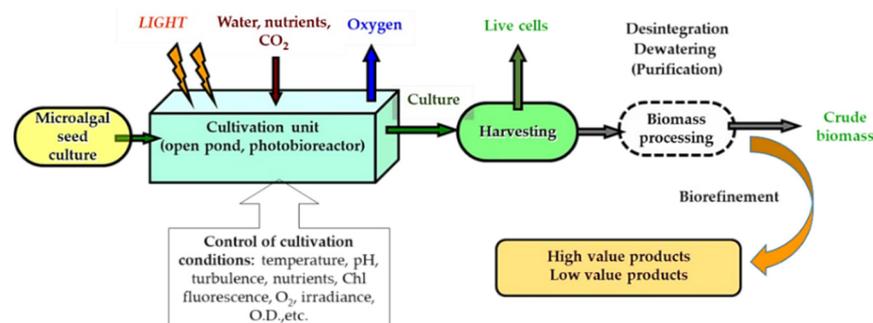


Figure 3. Scheme of microalgae cultivation and downstream processing options.

After cultivation, some filamentous and large-cell strains can be separated by gravity sedimentation or filtration using vibrating screens. Smaller cells can be harvested by centrifugation, filtration, or flocculation.

The selection of the harvesting method depends on the size, shape, and robustness of the organism as well as on the required quality and quantity of the product [66]. Moreover, the density of the culture suspension needed for subsequent processing steps is decisive [66]. In essence, the same techniques as for solid–liquid separation of digestate are used (see Section 4). If the culture is thin with small cells, a two-step procedure is needed, for example, filtration as a pre-concentration step followed by centrifugation [67]. This may be reflected in the price of the final product. Depending on the purpose of utilization, there are various options of consecutive steps after harvesting. Washing the biomass and/or extracting the valuable compounds from the biomass (with or without previous disintegration of the cells) might be suitable. For separating digestate components from biomass, membrane processes might be used, if those differ in size from microalgal cells.

### 6.1. Harvesting Techniques

Harvesting microalgae is challenging as the cultures are usually thin (dry weight  $\approx 0.5 \text{ g L}^{-1}$ ) and the cells are small (5–20  $\mu\text{m}$ ) [67]. There is no universal harvesting technique; however, even though it is cost- and energy-intensive, centrifugation is the most commonly used method for harvesting large volumes [66,67]. It is mainly applied when high-value products are aimed for [67]. Centrifugation is suitable for most types of microalgae, excluding some fragile species [66,68]. Various centrifuge types are available, e.g., tubular bowl, disc-stack, and scroll decanter [66]. Depending on the particle size, biomass can be concentrated up to  $150 \text{ g L}^{-1}$ . Most recently, the spiral plate gravitational

technology of Evodos was introduced, which allows to prepare microalgae cells in the form of a paste suitable for lyophilization [67,69]. Continuously operating centrifuges such as plate separators or nozzle separators are suitable for harvesting high amounts of microalgae for feed or aquaculture applications [66].

Flocculation usually means the collection of cells as aggregates caused by the addition of some chemicals, or coagulants (multivalent cations, metal salts, or polymers, for example  $\text{FeCl}_3$ ,  $\text{Al}_2[\text{SO}_4]_3$ ,  $\text{Fe}_2[\text{SO}_4]_3$ , polyethyleneamine, and chitosan) [66]. Thus, the particle size is increased, which makes it easier to separate them from the culture suspension [67]. Autoflocculation by pH change, or co-flocculation of co-cultures of particular microalgae and bacteria were also successful in some cases [67]. Flocculation is necessary to enhance the efficiency of biomass harvesting, which is especially important at a large scale [66]. The only drawback of flocculation is a potential unsuitability of the flocculant for the desired application or final product, or non-feasibility of scaling up [66,67]. Considering the use of microalgae for low-value products such as bioenergy, flocculation combined with sedimentation might be a more economically and energetically feasible harvesting technique than centrifugation.

Recently, new harvesting technologies, e.g., electrocoagulation and magnetic separation, were tested for cell separation (e.g., [70–73]). The former technique is usually based on coagulation, which is caused by electro-solubilization of iron or aluminum electrodes, but no metal ions remain in the medium [66]. Magnetic separation (the capture of microalgae cells by magnetic particles of  $\text{Fe}_3\text{O}_4$ ) has been used for removing microalgae in short operation time, but the practical application of this technique is still limited [66].

Another harvesting technique is filtration, which relies on porous membranes [66]. One advantage of filtration is that shear-sensitive species can be harvested without the addition of chemicals that could contaminate the product [66]. Microfiltration is suitable for harvesting biomass, and ultrafiltration can be used for the removal of metabolites [66].

When using digestate as a nutrient source, even when most particles were removed during digestate processing, some digestate components (e.g., suspended matter, salts) are still present in the cultivation liquid. Thus, when flocculation and centrifugation are used for harvesting, digestate components and flocculating agents remain in the separated microalgae biomass. This limits potential product applications. Filtration might be able to remove digestate components from the biomass but cleaning the filtration unit is linked to additional costs.

### 6.2. Disintegration of Cells

The disintegration of microalgae cells (disruption of the cell walls to release intracellular compounds) is an important operation in biomass processing as it concerns the preparation of intracellular products [66]. Physical-mechanical methods include freeze–thaw cycles, bead mills, high-pressure homogenization, and ultrasonication [74]. Alternatively, addition of chemicals (e.g., ionic liquids, enzymes) can lead to the disintegration of the cells [74]. The method for cell disruption depends on the toughness (i.e., composition) of the microalgae cell wall and required quality of the desired product [66]. Bead mills seem to be a good alternative for breaking microalgae with tough cellulose cell walls such as *Chlorella* or *Haematococcus* [66].

### 6.3. Dehydration of Cells

Depending on the product, dehydration of microalgal biomass might be necessary [75]. Following harvesting (and disintegrating) the microalgae culture, the 50- to 200-fold concentrated slurry (5–15% dry weight) must be processed quickly [66]. Dehydration of the microalgae slurry is achieved by solar or spray drying, or lyophilization [66,75]. Spray drying is a rapid method that yields a powdered product [66]. Lyophilization is the mildest of biomass drying methods since it is based on water sublimation from frozen biomass under vacuum and causes little damage to organic materials [75]. Both methods are expensive and are therefore only used for high-value products [66]. For low-value products,

solar drying is a cheaper alternative, but it requires large areas, might damage organic compounds, and is weather-dependent [75].

#### 6.4. Purification of Products

Various extraction techniques can be carried out to selectively remove a desired valuable substance (high-value products such as pigments, fatty acids, proteins, and polysaccharides) from the biomass into a liquid fraction [66,76]. Examples are solid–liquid extraction, microwave extraction, and ultrasound-assisted extraction [76]. Chromatographic techniques are most suitable for consecutive product fractionation and purification [66]. In the course of extracting and purifying certain substances, remaining digestate particles are removed. This is favorable and would increase the product quality. However, considerable costs are associated with these processes. The residual biomass can be used as low-value product for industrial or agricultural purposes (for biofuels, aquaculture fodder, or biofertilizers, see also Section 7) [66].

### 7. Use of Microalgae Biomass

The amount of digestate has been rising in industrial processes. It can be declared as a by-product rather than waste (Directive 2008/98/EC [77]) and thus the utilization of anaerobic digestate of industrial side streams is beneficial. The declaration as by-product makes it easier to utilize microalgae products and enlarges their area of application. Therefore, valuable products can be obtained from microalgae that were cultivated on digestate medium [78]. Additionally, upstream costs of the process can be lowered by using nutrients present in the digestate [38].

Nevertheless, legislative issues must be considered when an environmentally and economically favorable process is designed to assure that quality requirements of certain products are met [78]. The aim should be microalgal biorefinery, which means that the produced biomass is used for multiple products and wastes are avoided [78]. Valuable components such as pigments and storage components can be extracted before using the residual biomass as animal feed or converting it anaerobically or thermally to bioenergy [79].

#### 7.1. Bioenergy Production

Like biomass produced using mineral media, biomass grown on digestate can be used for bioenergy production (e.g., biodiesel, biogas, and bioethanol) [78]. Of those, biodiesel has been studied most thoroughly [78,80]. The accumulation of high amounts of lipids under nitrogen deprivation, among other triggers, makes microalgae a promising feedstock for biodiesel production [80]. The fatty acid profile of the biomass determines the quality of the obtained biodiesel [78]. Using digestate as nutrient source affects not only the lipid content but also the fatty acid pattern of microalgae [78]. While high amounts of ammonia in pure digestate inhibit microalgae growth and negatively affect lipid accumulation, several studies have shown that diluted digestate is a suitable nutrient source that leads to increased lipid and biomass production [78].

Moreover, biomass derived from numerous strains (e.g., *Dunaliella salina*, *Scenedesmus obliquus*, *Spirogyra* sp.) is a valuable feedstock for bioethanol production as it contains significant amounts of carbohydrates (up to 64% TS) when grown under stress conditions [78,81]. It has several advantages over conventional feedstocks (food crops, lignocellulosic materials) [78,81]. The biomass does not contain lignin, which means that pretreatment is easier [81]. Instead, starch is present at high amounts, which can easily be converted to sugar monomers [78]. Even though bioethanol production based on microalgae grown on digestate medium has not been studied extensively yet, it has been shown that the carbohydrate content of the obtained biomass is similar to that of microalgae grown on mineral medium [78].

Furthermore, microalgae biomass represents a promising organic source to produce biogas [82]. Anaerobic digestion of *Acutodesmus obliquus* biomass obtained a CH<sub>4</sub> yield of

200–391 Nm<sup>3</sup> t<sup>-1</sup> VS, and the maximal yield was achieved through digestion of biomass with increased lipid content (+20%, in total 30% TS, due to nitrogen deprivation) [37]. *Synechocystis* cf. *salina* resulted in 429 Nm<sup>3</sup> t<sup>-1</sup> VS and 348 Nm<sup>3</sup> t<sup>-1</sup> VS prior and after the extraction of valuable compounds (polyhydroxybutyrate), respectively [79]. These values are comparable to maize silage (340–360 Nm<sup>3</sup> t<sup>-1</sup> VS), a commonly used feedstock for biogas production [83]. The digestion of microalgae together with other substrates (co-digestion) has been investigated in various studies [82,84,85], and positive synergies are formed (e.g., improved C/N ratio, reduced viscosity) [82,84]. However, there are seasonal variations in the availability and composition of microalgal biomass and co-substrates that affect biogas yield. Additionally, microalgae can be used for biogas upgrading to remove CO<sub>2</sub> [78]. In summary, microalgae that were grown on digestate can also be used in other stages of biogas production.

For biodiesel or bioethanol production, purity requirements of biomass are low, as lipids or carbohydrates, respectively, are extracted from the biomass. This means that residual constituents of digestate will be removed during this process and are not harmful. When used as substrate for biogas production, residual digestate could even be an advantage, as it might introduce beneficial trace elements. Therefore, microalgae grown on anaerobic effluents can contribute to reducing the costs of producing these low-value products.

### 7.2. Applications in the Food and Feed Industry

Microalgae such as *Chlorella*, *Arthrospira*, *Haematococcus*, and *Dunaliella* are commonly used in foods, e.g., as human nutritional supplements, due to their beneficial properties (e.g., antiviral, antitumor, anti-inflammatory) [86]. They are a rich source of proteins, carbohydrates, healthy lipids, and antioxidants such as pigments [86].

Microalgal biomass produced on digestate has high potential to be used in aquaculture. Live microalgae can be fed directly to fish larvae, mollusks, or crustaceans, or to rotifers that can be a live feed for fish or crustacean larvae—as aimed for in the Algae4Fish project (Interreg ATCZ221). Another possibility is to use refrigerated or frozen pastes of microalgae (*Chlorella*, *Nannochloropsis*, *Phaeodactylum*), which are fed to herbivorous fish as well as larvae of shrimps, oysters, sea bream, bivalves, abalone, crab, or zooplankton. Spray-dried, disintegrated, or freeze-dried products are available as intact cells or with broken cell walls, fed to zooplankton, algae grazers, or mollusks, or mixed (1–5%) in expensive commercial feed preparations.

However, new products entering the food chain must meet requirements concerning public health, hygiene, and safety [78]. In the European Union, the General Food Law (EC 178/2002 [87]) sets the basic legislative framework for food and feed production [86]. Regulations such as Hygiene Regulations (EC 852/2004 [88], EC 853/2004 [89]) and the Legislation about Novel Food (EC 258/97 [90]) must be followed when producing microalgae for nutritional applications [78,86]. EC 767/2009 [91] comprises specific regulations for new ingredients for feed purposes [78,86]. The level of contaminants in microalgal biomass must be tested before it is used as animal feed (2002/32/EC [92]) [78,86].

### 7.3. High-Value Products and Microalgae Biorefinery

A biorefinery approach can increase the sustainability of microalgae production by exploiting the biomass more completely and avoiding waste [78]. As an example, the SABANA project (EU Horizon 2020 Research and Innovation Program under grant agreement no. 727874) aims to demonstrate a large-scale microalgae-based biorefinery concept for the production of high-value products (biostimulants, biopesticides, aquafeed additives) as well as low-value products (biofertilizers, fish feed), recovering nutrients from wastewaters (sewage, supernatant, and pig manure) in continuous mode all year round [13,93].

Biohydrogen production can also be an approach to create a microalgal biorefinery. During the dark fermentation process, anaerobic microorganisms convert biomass into hydrogen and CO<sub>2</sub> [94]. The arising effluent can serve as nutrient source for mixotrophic microalgae cultivation, similar to digestate [94]. The byproduct of the first stage, CO<sub>2</sub>,

might be introduced as a carbon source. Provided that the obtained biomass has high carbohydrate content, it would be a suitable feedstock for dark fermentation [95]. Thus, the microalgal biomass can be returned into the first stage of the process, serving as substrate for hydrogen production [94]. However, the concept of biohydrogen production using microalgae is still at an early stage.

Another biorefinery model, based on digestate, focuses on the production of the biodegradable biopolymer PHB, which can replace commercial plastics such as polyethylene and polypropylene. It is produced by several prokaryotic microalgae (cyanobacteria) such as *Nostoc*, *Arthrospira*, and *Synechocystis* under phototrophic or mixotrophic growth and nutrient-limited conditions [96]. Recently, digestate was investigated as a low-cost substrate for cultivating *Synechocystis* cf. *salina* for PHB production [97]. In mineral medium, PHB accumulation was induced by nitrogen limitation, which led to consumption of phycocyanin and orange coloring of the biomass. This was not observed when digestate was used. Therefore, it is suggested that phycocyanin and PHB can be obtained as products. Besides that, the residual biomass can be used as animal feed or anaerobically converted to biogas.

In addition to phycocyanin, microalgae are a biological source for several other valuable pigments [98]. Besides their utilization as natural colorants, they have various potential application areas such as food industry, feed additives (e.g., aquaculture), and cosmetics due to their high nutritional and bioactive value [78,98]. It has been shown that the cultivation of *Chlorella vulgaris* and *Scenedesmus* in diluted digestate leads to carotenoid accumulation [78]. Moreover, the limited light permeability of diluted digestate can cause higher chlorophyll and phycocyanin contents compared to cultivation in transparent mineral medium [78]. Thus, pigment extraction of microalgal biomass should receive attention to make microalgal biorefineries with digestate as nutrient source more economically feasible [78]. However, it is questionable if waste streams such as digestate can be used for the production of compounds for human use [98].

The microbial production of exopolysaccharides (EPSs) can be applied for natural products, whose demand is rising because they are used in a wide range of biotechnological, medical, and industrial applications [66]. Further research is necessary to investigate the effect of using digestate as nutrient source on EPS production.

Microalgae biomass grown in digestate media can be applied as environmentally friendly slow-release fertilizer [78]. This application can be an addition to the use of the solid digestate fraction as fertilizer, utilizing the nutrients of the effluent in a more complete way. In a biorefinery approach, this can serve as final valorization of the remaining biomass after extracting other valuable compounds such as PHB, lipids, or pigments.

## 8. Thoughts on Economics and Sustainability

Microalgae cultivation requires several energy- and cost-intensive steps, especially during downstream processing. If digestate is used as a nutrient source, additional environmentally and economically costly operations might be necessary to make it suitable as a cultivation medium. At the same time, the need for the production and acquisition of inorganic nutrients can be omitted by using a nutrient-rich waste stream instead [99,100]. Using nutrients, water, and CO<sub>2</sub> from such cheap sources can reduce production costs by 50% and simultaneously improve sustainability [100,101]. The required treatment depends on the digestate, microalgae species, and product. Therefore, it must be evaluated for each individual case if the usage and the associated treatment of digestate are suitable. If elaborate processing steps or the partial addition of inorganic nutrients were necessary, drawbacks would outweigh the benefits [98].

Nonetheless, in spite of potentially higher upstream costs, the proposed concept can be beneficial if microalgae production is incorporated into an existing process [102]. As mentioned, the water content of digestate must be reduced for its application as fertilizer in agriculture. Biofertilizer production from digestate often includes processes that would also be needed for making the raw digestate suitable for microalgae cultivation

(e.g., solid–liquid separation, ammonia stripping, struvite precipitation). Consequently, it may yield a waste fraction that is exactly suitable for microalgae production [102]. This nutrient-rich liquid fraction is usually processed in a wastewater treatment plant and disposed, which requires large amounts of chemicals and energy [99]. As stated by Acien et al., wastewater treatment costs could be reduced by 17% compared to activated sludge treatment by applying microalgae technology with the additional production of biofertilizer and biomethane [98]. Thus, obtaining the nutrient-rich waste fraction for microalgae production would be beneficial for both processes.

However, large amounts of energy are needed for downstream processing, as described above [99]. Methods such as gravity sedimentation and solar drying should be used whenever necessary to minimize the costs [98]. Moreover, it is essential that the whole process not only serves as effluent treatment. The produced biomass should be valorized and multiple products obtained by applying a biorefinery concept [98,99]. A positive energy balance can be achieved if the biomass is used for the production of bioenergy [98].

## 9. Concluding Remarks and Future Perspectives

To obtain optimal productivity, the composition and consistency of digestate must be suitable. These strongly depend on the feedstock and parameters used in anaerobic digestion. Digestates from certain biogas production processes might be better suited for microalgae cultivation than others; thus, also the anaerobic digestion itself must be considered during process development. Digestate composition, especially when received from an industrial biogas plant, hardly changes during seasons. This should allow stable process conditions as well as consistent product quality. However, the raw digestate needs to be processed prior to its use in microalgae cultivation in order to adjust total solids and nutrients. At the very beginning of the process chain, it is important to already have the desired product in mind. Product price and quality requirements might determine the necessary and possible amounts of resources spent on digestate processing. Since the aim is to use digestate as sustainable and economically advantageous option, the effort of digestate treatment as well as the application of chemicals and use of energy should be kept as low as possible, especially for low-value products and bioenergy.

Considering a biorefinery approach, the aim should be to use all side fractions that arise during digestate treatment and microalgae processing. The use of the solid phase of digestate as fertilizer enables synergies with agriculture. Residues arising during microalgae downstream processing (process water, residual microalgae biomass) can be recycled into the microalgae cultivation or biogas production process. As a consequence, a suitable combination of digestate treatment as well as downstream processing steps must be applied. This way, waste and loss of valuable resources can be avoided. Simultaneously, a suitable and sustainable cultivation medium and, above all, marketable microalgae products with adequate quality may be obtained. Depending on the application, the quality requirements differ, and additional separation or washing steps might be necessary to remove remaining digestate substances for high value products or applications where the whole biomass is used. Additionally, legislative issues must be considered, especially for the use in the food industry. Aquaculture, agriculture, and biofuels are large markets with medium, low, and almost no safety requirements, respectively. These markets are therefore interesting for microalgae products derived from cultivation in digestate.

The use of digestate as nutrient source for microalgae cultivation is advantageous when considering the environmental impact. However, only a few studies dealing with downstream processing of microalgal biomass grown in digestate media compared to mineral media are available. Further research must be conducted to identify the most suitable digestate processing methods and downstream techniques. Moreover, the biomass of algae grown in diverse digestate media should be characterized in order to determine suitable application areas. In this regard, both beneficial and harmful substances must be identified. The most important parameters that affect the productivity of the process must be identified and adapted in a way that leads to lowest possible production costs.

Finally, digestate treatment and microalgae downstream processes should receive increased attention because they represent major steps in the production process.

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