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Review

Algal Biomass from Wastewater and Flue Gases as a Source of Bioenergy

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Abstract: Algae are without doubt the most productive photosynthetic organisms on Earth; they are highly efficient in converting CO_2 and nutrients into biomass. These abilities can be exploited by culturing microalgae from wastewater and flue gases for effective wastewater reclamation. Algae are known to remove nitrogen and phosphorus as well as several organic contaminants including pharmaceuticals from wastewater. Biomass production can even be enhanced by the addition of CO_2 originating from flue gases. The algal biomass can then be used as a raw material to produce bioenergy; depending on its composition, various types of biofuels such as biodiesel, biogas, bioethanol, biobutanol or biohydrogen can be obtained. However, algal biomass generated in wastewater and flue gases also contains contaminants which, if not degraded, will end up in the ashes. In this review, the current knowledge on algal biomass production in wastewater and flue gases is summarized; special focus is given to the algal capacity to remove contaminants from wastewater and flue gases, and the consequences when converting this biomass into different types of biofuels.

Keywords: algae; biofuels; CO_2; flue gases; nitrogen; phosphorus; wastewater

1. Introduction

World energy demand is increasing continuously due to industrialization, technology development and population growth, and is expected to reach 9.2 billion by 2050 [1]. In 2015, the world's total primary energy was supplied by fuel petroleum (that accounted for 31.7%), coal (28.1%), natural gas (21.6%), biofuels and waste (9.7%), nuclear (4.9%), hydro (2.5%) and the combination of geothermal, solar, wind, tidal/wave/ocean, heat and others (1.5%) [2]. A continuous dependence on fossil fuels will raise energy prices as crude oil reserves diminish. Energy based on fossil fuels might, therefore, be economically unsustainable in the future [3], but even more important is its footprint on our climate: worldwide reductions in global greenhouse gas emissions (GHG) will be essential to avoid the worst consequences. GHGs are the main cause of global warming and melting of the poles, and they also lead to the loss of marine biodiversity through ocean acidification. The oceans have been found to absorb approximately one-third of the anthropogenic carbon dioxide (CO_2) emitted each year, which then is dissolved and gradually reduces the pH of the water [4]. Thus, the use of renewable energy is essential if we want to sustain the Earth for future generations. However, to be a viable replacement for fossil fuel, the source of renewable energy has to be economically competitive, should be produced at amounts able to meet energy demand, and its production has to provide a net energy gain [3]. By 2020, the European Union (EU) countries are required to provide 20% of the final energy consumed from renewable sources [5]; 56% of the renewable energy supply in the EU should originate from biomass [6]. The Energy Strategy 2020 of the European Commission has given a long-term target for the EU and other industrialized countries resulting in an 80–95% cut of GHG emissions by 2050 [7]. Additionally, the United Nations Paris Agreement proposes a worldwide reduction to 40 Gt CO_2 emission equivalents instead of the 55 Gt aimed at by 2030.

The term biomass covers all organic material in non-fossil form originating from land- and water-based vegetation as well as all organic wastes [8]. Biomass is a complex mixture consisting mostly of carbohydrates, lipids, lignin, proteins and organic acids [9]. Contrary to fossil fuels, biomass is a source of clean energy with a negligible content of sulfur, N (nitrogen) and ash, resulting in low emissions of sulfur dioxide (SO_2), nitrogen oxides (NO_x) and, importantly, the CO_2 emission is, at least, theoretically net-zero [10]. Nevertheless, gaining energy from biomass feedstock, originating from agriculture, forest lands or other ecosystems, has ecological and economic impacts [11,12]. The biomass is usually either burnt to produce electricity and thermal energy, or used for the production of transportation fuels, so-called biofuels [13]. Biofuels are generally classified in three categories: (i) first-generation biofuels originate from biomass that is generally edible (i.e., food-crop feedstock), mainly consisting of sugar, starch, and vegetable oil; (ii) second-generation biofuels are bio-based products that come from non-food feedstock, such as lignocellulosic agricultural, forest feedstocks and municipal solid wastes; and (iii) third-generation biofuels are produced from microalgal biomass [14]. The expansion of first-generation biofuels and their direct competition with food-crop production for land area has negatively impacted the environment; GHG emissions were actually raised from changes in land usage and biomass-conversion systems; pollutants were emitted and water depleted [3,15–18]. Not only did biodiversity decrease, but also global food prices increased dramatically [12,19]. The production of second-generation biofuels, instead, has the prospective advantages of consuming waste residues and using abandoned land, thus promoting the improvement of economic conditions in emerging and developing regions. However, currently, the production of such fuels is not cost-effective, and technical hitches have to be resolved before their potential can be realized [20,21]. The growth of second-generation biofuel crops could also become unsustainable on a long-term basis when they compete with the plantation of food crops for available land [22]. For both the production of first- and second-generation biofuels, large areas are needed to cultivate the crops, and hence they are not a viable alternative to fossil fuels, as the produced volumes cannot cover our global fuel demand [23,24]. Therefore, the exploitation of microalgae to generate third-generation biofuels seems to be a feasible alternative for producing renewable energy, overcoming the disadvantages of first- and second-generation biofuels [25,26].

Microalgae have much higher growth rates and productivities than conventional forest or agricultural crops and other aquatic plants. They can synthetize lipids in concentrations up to 70% dry-weight biomass, and exposed to nutrient- or other environmental stress their lipid amounts can even be up to 90% [25–28]. Microalgae have a very short harvesting cycle compared to crop plants, which are generally harvested once or twice per year; plus, microalgae can grow all year round (depending on species and cultivation conditions), allowing several continuous harvests with significantly increased yields [29]. Thus, the yield of microalgal oil per area may substantially exceed the yield of the best oilseed crops [25,30]. It is estimated that microalgae can produce up to 94,000 L biofuel per ha per year, while corn only yields about 560 L per ha annually [31]. Moreover, the aquatic growth of microalgae in tanks or bioreactors requires less land area: up to 297 or 770 times less than soybean or corn crops, respectively [25]. Microalgae require less freshwater than terrestrial crops, which reduces the pressure on freshwater sources. Depending on the species, they even can grow in marine, brackish and/or wastewater, which are unsuitable for human consumption. Microalgal cultures do not require the application of herbicides or pesticides. Different species are adapted to live in a broad variety of environmental conditions, allowing culturing in various environments, contrary to terrestrial feedstocks [30,32]. In addition, different types of renewable fuels such as biodiesel, methane, hydrogen or ethanol can be produced from microalgal biomass [25,30,33]. Biodiesel from microalgal biomass has been shown to perform as well as petroleum diesel, while at the same time its emissions of particulate matter, CO, hydrocarbons, and SO_x are reduced [34]. Some microalgae species even produce high-value compounds, such as polyunsaturated fatty acids, natural dyes, polysaccharides, pigments, antioxidants, bioactive compounds, and/or proteins, which can potentially revolutionize the industries of cosmetics, pharmaceuticals, nutrition, food additives and aquaculture [26].

From an ecological and economic perspective, it is particularly interesting the possibility of culturing microalgae in wastewater, allowing the bio-treatment of sewage coupled with the production of potentially valuable biomass, and at the same time reducing GHG emissions [35]. Microalgae are able to take up ammonium, nitrate and phosphate from wastewater. As photosynthetic organisms, they further are able to transform CO_2 from flue gases into biomass [36], thereby reducing CO_2 emissions, the major GHG. With our current technology, large-scale biofuel production from algae seems only to be economically feasible when coupled with wastewater treatment [37]. The production costs of algal biomass using a 100 ha open pond system were estimated to be €4.95 per kg [38]. Even with an all-year operation and the optimistic assumption of lipids to be 35% of the biomass [39], the price of crude algae biomass currently is still not economically feasible. However, combining algal biomass production with tertiary wastewater treatment will create better economic prospects. Many reviews combine the current knowledge on the topic; in this review, we will, therefore, focus only on the latest literature. Wastewater as culture medium is a cost-effective solution for recycling nutrients, and the need for additional N and P (phosphorus) sources is estimated to decrease by about 55% [40]. This review will focus on our current knowledge about culturing microalgae in different types of wastewater and flue gases, the various microalgal species adapted to grow in wastewater, and it will summarize research focusing on the conversion of algal biomass.

2. The Composition of Wastewater and Flue Gases

Depending on its source, wastewater is classified as municipal, agricultural or industrial. Although microalgae have been used for wastewater treatment since the late 1950s [41], at present the chemical processing of wastewater or the manufacture of activated sludge are still the most common wastewater-treatment methods [35]. Removal of N and P is an important requirement of wastewater treatment; otherwise, if discharged to water systems, these nutrients will cause eutrophication of aquatic ecosystems, potentially endangering human health, biodiversity and ecosystem sustainability [42,43]. Nitrogen is present in the wastewater influent as ammonia (NH_4^+), organically bound nitrogen or even nitrite (NO_2^-) or nitrate (NO_3^-). Phosphorus is present in both the wastewater influent and effluent primarily in the form of phosphates (PO_4^{3-}). Although municipal and industrial wastewaters are usually treated before their release into the environment, agricultural wastewater derived from animal manure is often directly spread over feedstock lands as fertilizer, and it can contain N and P concentrations of >1000 mg L^{-1} [44]. Crops are not able to fully absorb the nutrients provided by the manure, and thus the excess nutrients will be washed off, resulting in eutrophication of receiving waters. Agricultural runoff can further contain herbicides, fungicides and insecticides. Municipal wastewater can also contain multiple inorganic substances from domestic and industrial origin, comprising several heavy-metal pollutants such as arsenic, cadmium, chromium, copper, lead, mercury and zinc [45]. Compared to agricultural and municipal wastewater, there is less N and P in industrial wastewater. On the other hand, industrial wastewater may contain high levels of heavy-metal pollutants and organic chemical toxins (hydrocarbons, biocides, and surfactants) [46]. Nevertheless, its composition is extremely variable depending on the industrial sector.

Commonly, wastewater treatment is performed in two or three stages as follows: (i) the primary treatment removes large particles through grids or sedimentation; (ii) the secondary treatment diminishes the biochemical oxygen demand (BOD) by oxidizing organic compounds and NH_4^+. This treatment is often carried out in aerated tanks using bacteria that degrades the organic material and protozoa that grazes on the bacteria, and it results in activated sludge. The organic material is then converted to CO_2 and water; (iii) tertiary wastewater treatment, i.e., the last treatment prior to discharging the water into the environment, removes mainly the inorganic compounds including N and P [47]. N is usually removed in a two-step-process, including nitrification and denitrification; NH_4^+ is first oxidized to nitrate by nitrifying bacteria in aerobic reactors, and thereafter in anoxic reactors it is converted to nitrogen gas (N_2) by denitrifying bacteria. Removal of P is achieved by chemical precipitation with aluminum or iron salts to form aluminum or ferric phosphate. These salts are usually added in excess to overcome natural alkalinity [47]. Alternatively, P is adsorbed to filter materials containing positively charged surfaces [48] or taken up by bacteria exposed to alternating aerobic and anaerobic conditions in a process called enhanced biological phosphorus removal [47]. Unfortunately, removed N and P cannot be fully recycled, and instead are buried in landfills or further treated to generate fertilizers [49].

Both the production of fertilizers for feedstock cultivation, and the remediation of wastewater where the remaining of fertilizers end up, have high capital and energy costs [11,50] resulting in a waste of nutrients. This is of major concern as the industrial production of fertilizers relies heavily on N from the energy-intensive Haber–Bosch process (which consumes around 1% of global energy production); P is derived from the mining of phosphate rock, a non-renewable activity that is estimated to lead to depletion of the Earth's phosphorus supply within 50–100 years [51]. Microalgae used for the treatment of wastewater, therefore, provide an excellent alternative, because these photosynthetic organisms assimilate N, P and C during their growth, the nutrients can then be recycled and used as bio-fertilizer. The inorganic nutrients present in wastewaters provide cost-effective media for microalgal cultivation and at the same time recycle water, which reduces the overall water footprint of biofuel production from microalgal biomass [26,37,40]. Contrary to conventional wastewater treatment, the treatment of wastewater with microalgae does not create additional pollution, and therefore it offers an ecologically safe, cheap and efficient way of removing nutrients [52].

The concentrations of N and P present in wastewater differ considerably depending on its type and the stage of its treatment process [53]. Municipal wastewater (also called sewage) has relatively low amounts of total N and P; concentrations are around 10–100 mg L^{-1} [44]. After secondary treatment, total N and P diminish to ranges of 20–40 mg L^{-1} and 1–10 mg L^{-1}, respectively [54]. These concentrations are highly suited for microalgal cultivation. Sewage contains N and P in a ratio of 11 to 13 (calculated on the basis of NO_3^- and PO_4^{3-}) [55]. An N:P ratio of 16 originally was widely accepted as optimal for algal growth [55–58], based on the empirical formula $C_{106}H_{181}O_{45}N_{16}P$ describing the chemical composition of algae [59]. The typical algal biomass contains 6.6% N and 1.3% P in dry weight [60] with a molar N:P ratio of 11.2, a composition similar to that found in wastewater. Based on the N and P content found in biomass, one can assume that algae cultures in wastewater can produce 0.3–1.15 g L^{-1} of dry weight. Following this assumption, each kilogram of algae produced in wastewater should have assimilated approximately 66 g of dissolved N and 13 g of P, corresponding to 0.7–3.0 m^3 of cleaned wastewater. At low N:P ratios, microalgae are able to assimilate P in excess (luxury uptake); the nutrient is then stored within the cells inside the granules in the form of polyphosphate [56,61] and can be metabolized in case of P limitation. Luxury uptake of P in algae increases their cleaning capacity of wastewater. Nitrogen limitation in the culture medium can further lead to N starvation, a recognized stress factor used to promote lipid accumulation in algae [27,32,62,63]. The growth of algae in wastewater, therefore, allows the recycling of N and P ions and at the same time it is an effective way to produce biofuel at decreased cost [35].

Flue gases from burning natural gas and coal contain 5–6% and 10–15% CO_2, respectively [36,64]. In addition to CO_2, flue gases may contain more than 100 different compounds, including several noxious gases such as nitrogen oxides (NO_x, with nitric oxide and nitrogen dioxide being dominant) and SO_x, as well as N_2, H_2O, O_2, unburned carbohydrates (C_xH_y, such as methane and butane), CO, heavy metals, halogen acids and particulate matter [65].

In order to decrease CO_2 emissions from flue gases, several CO_2 sequestration strategies have been implemented globally. Carbon capture and storage (CCS) methodologies have been widely and intensely studied, and take place in three steps: CO_2 capture, CO_2 transportation and CO_2 storage. CO_2 is stored by absorption, adsorption, gas-separation membranes, or cryogenic distillation. This CCS process is usually carried out at facilities that discharge large amounts of CO_2 into the atmosphere (e.g., cement-manufacturing facilities and power plants) daily [66,67]. The gas mixture rich in CO_2 is compressed to a supercritical fluid or liquid, which is then transported and stored in deep oceanic or geological trenches, or is mineralized [68]. Although CCS methodologies meet their objectives, they are only short-term solutions with numerous challenges associated, i.e., high costs, energy and space requirements and potential CO_2 leakage over time. Rock-weathering enhancement is a method relying on physical and biological properties to reduce atmospheric CO_2; however, there is uncertainty about its time range. Alternatively, biological strategies to capture CO_2 are based on enhancing photosynthesis on our planet either by CO_2 afforestation, ocean fertilization, or microalgal cultivation [69]. These processes are an economically and environmentally viable option to be explored, considering that photosynthesis not only captures CO_2, but even releases oxygen as a side product. Although terrestrial plants are able to sequester large amounts of CO_2 from the atmosphere, photosynthesis performed from forests only accounts for 3–6% of carbon annually fixed on the planet, whereas seagrass, algae and cyanobacteria are responsible for approximately 40% [70]. Due to the great photosynthetic efficiency of microalgae, which is 10 times higher that of terrestrial plants, their cultivation provides the most promising and environmentally sustainable biological CO_2 capture process [36].

3. Microalgal Wastewater Reclamation and Biomass Production

Successful microalgal cultivation on an industrial scale using wastewater and flue gases will depend on the selection of excellent microalgal strains from either natural screening or genetic engineering. Strains isolated near power or wastewater-treatment plants may be more tolerant than other native species. One should keep in mind that different species vary considerably in tolerance and productivity. Tedious selection procedures are required depending on the location of the algal farm, the origin of wastewater, and the purpose of the biomass. Many excellent recent reviews summarize the use of various microalgae; in this section, we therefore will refer only to the most recent publications.

3.1. Algal Growing Regimes and Physiology to Increase Target Macromolecules

The common term algae refers to a large group of aquatic photosynthetic organisms, and includes eukaryotic macro- and microalgae as well as prokaryotic cyanobacteria. The major algal groups are the following: cyanobacteria (Cyanophyceae), green algae (Chlorophyceae), diatoms (Bacillariophyceae), yellow-green algae (Xanthophyceae), golden algae (Chrysophyceae), red algae (Rhodophyceae), brown algae (Phaeophyceae) and dinoflagellates (Dinophyceae). Green algae are, without any doubt, the most important group of algae used in various wastewater treatment processes. This Chlorophyta phylum includes about 6000 genera of approximately 10,000 species of microscopic and macroscopic eukaryotic algae. All these species contain chloroplasts surrounded by two envelope membranes and an internal membrane called thylakoids, which is partly stacked (grana); the photosystems located inside the thylakoid membrane contain chlorophyll *a* and *b*. Starch deposits inside the plastids are the main polysaccharide reserve [71]. The majority of species used in wastewater-treatment processes belong to the genus *Chlorella* and *Scenedesmus*, which are particularly tolerant to harsh environment and high level of nutrients.

Based on the use of energy and carbon source, algae are able to photoautotroph, heterotroph, photoheterotroph or mixotroph growth. In photoautotroph growth, algae use light as an energy source and inorganic carbon as a carbon source; during heterotroph metabolism the source of energy and carbon are organic compounds. Photoheterotroph growth allows light to be the energy source and organic carbon the source of carbon; and during mixotroph metabolism, algae can use both light and organic compounds as a source of energy and both inorganic and organic carbon as a source of carbon [72]. Depending on the chosen growth regime, the algal biomass composition will differ [73], which should be considered when specific macromolecules such as lipids or carbohydrates are the target products. Commonly for the production of biodiesel a high amount of algal lipids is needed, while biomass with a high portion of carbohydrates is important for the production of bioethanol, biobutanol and biohydrogen.

In some studies the mixotrophic growth in the presence of both organic and inorganic carbon has been shown to be the most productive for achieving a high algal growth rate and final biomass [72,74,75]. Hence, the organic and inorganic carbon present in wastewater and flue gases could potentially result in high biomass production during mixotrophic growth. Several cultivation methods have been developed with the aim of increasing the algal cell content with energy-rich macromolecules (lipids and carbohydrates).

Exposing the cells to stress has been shown to be highly effective at increasing the concentration of target macromolecules. A stress can be produced by altering either chemical or physical parameters in the culture. Chemical parameters usually include nutrients of the growth medium: carbon, nitrogen, phosphorus, sulphur, iron or salt stress (sodium chloride); while physical parameters include light or temperature [73].

The amount of CO_2 present as an inorganic carbon source affects the concentration and cellular localization of starch; a *Chlorella* strain grown at high CO_2 concentrations (3% in air) stored starch mainly in the stroma, while during growth at low CO_2 level (air level) starch was stored in the pyrenoids [76]. Hence, algae grown in the presence of flue gases that can contain considerably high amounts of CO_2 might be affected in their starch accumulation or cellular storage-localization.

Nitrogen deprivation is one of the most frequently used parameters to increase the lipid content [32], and carbohydrate content of algal cells [77].

Phosphorus limitation was shown to increase the lipid content in algal cells [32]; while P and sulphur limitation increased starch content in *Chlorella* cells, although in the case of P, this was only for a very limited period of time, after which it decreased again [77].

Some algal species accumulate carbohydrates at high light intensity [73]. However, we should bear in mind that algal bioreactors for wastewater treatment often use natural irradiation, which cannot be manipulated and is weather dependent. One way to influence natural light intensity available to the algae is by changing reactor configuration. In field trials, it has been shown that the increase of sunlight together with the limitation of nitrogen resulted in very high lipid productivity [32]. Temperature has been shown to affect the fatty acid composition of the lipids; low temperature stimulates the biosynthesis of unsaturated fatty acids [78] that improve fuel fluidity, but decrease its oxidation stability.

3.2. Microalgae Strains Used for Wastewater Reclamation

The majority of species used in wastewater-treatment processes belongs to the genus *Chlorella* and *Scenedesmus*, which are particularly tolerant. The genus *Chlorella* with more than 100 unicellular members is best studied among green microalgae; the unicellular algae consist of small round cells protected by a hemicellulose-containing cell wall, providing rigidity [79]. *Chlorella* species are fast growing, very resistant and effective in removing N and P from different wastewater streams [58]. The downside of using *Chlorella* species in wastewater treatment can be their small size and high stability in liquid cultures, which increases the costs of harvesting and biomass separation. *Scenedesmus* species perform very well in wastewater-treatment processes. The genus is comprised of approximately 70 colonial, green microalgae species; *Scenedesmus* cells are 5–8 μm wide, twice as long, and form 2 to 16 cell-colonies with poles of terminal cells equipped with a single spine [80]. Beside culture-collection strains, locally isolated strains and mixed cultures can be successfully implemented in wastewater treatment [81–83].

3.3. Monoculture Versus Community

An algal monoculture consists of a single strain precisely selected to produce a desired compound with maximal yield [84]. Algal monocultures cultivated in non-sterile systems like open ponds, using pretreated conventional culture medium, are susceptible to contamination by wild strains unless additional means of control are utilized [55]. In wastewater, which contains many microorganisms, it is very difficult to maintain the inoculated strain as a monoculture for longer periods before the culture will be overtaken by other algae present in the environment [55]. Therefore, monocultures of high lipid-producing strains are likely to be out-competed by faster growing species [85], depending on many environmental and biological parameters. Maintaining the dissolved oxygen (DO), a certain pH, the culture retention time and dilution rates at certain values are some of the possible solutions favoring the growth of the desired species. However, the reason why a certain algae strain is dominant over another is still not fully understood [55]. Locally isolated strains from wastewater ponds have been shown to perform better in wastewater treatment than strains from culture collections [81,82,86,87]. Also, algae consortia have been found to be more stable than monoculture [81,82]. The shift in the culture composition from selected species to a mixed culture will influence growth parameters as well as the expected final biomass concentration and its lipid content. However, one should keep in mind that mixed cultures also accumulate lipids; if biofuel is the desired product, the biomass production, therefore, might still be satisfactory [55]. In order to maximize the performance of each strain, the chemical composition of the wastewater should be analyzed in detail prior to its use as a medium for microalgal cultivation [87].

3.4. Control of Grazers

Not only microorganisms, but grazers are also very common in open pond cultures of wastewater. Grazers can be controlled through physical and chemical treatment. An application of e.g., 10 mg L^{-1} quinine chloride to a contaminated outdoor-growing algae mass culture can completely remove grazers within a short time [88]. However, in wastewater treatment any addition of chemicals is unsuitable for economic reasons as well as to avoid secondary water contamination. Adjusting the pH to a high value of 11 for a short period of time was suggested to be an efficient method to remove most grazers, especially in wastewater treatment, which already contains high levels of ammonia [55]. Obviously, the selection of robust algae strains is the best solution; in a mixed culture with invasive algae species, the high growth rates will enable the algae to withstand a low population of grazers (with much lower growth rates).

3.5. Enhanced Lipid Production During Wastewater Treatment

The total price of crude algal biofuel production using large-scale open-pond cultivation systems was estimated to be €24 per L ($109 per gal), which is far above the costs of producing commercial petrol diesel [89]. Profitable crude algal biofuel production will require the development of algae strains and conditions for culture that allow the rapid production of algal biomass with high lipid content and minimal growth of competing strains. Nevertheless, coupling the utilization of wastewater and flue gases to microalgae production is an important part of the development process, since it decreases production costs, provides wastewater bioremediation and reduces air contamination. Large-scale cultivation of algae for lipid and biofuel production requires the availability of space, water, fertilizers, sunlight and CO_2. The employment of a two-stage cultivation strategy as well as the use of a native, mixed culture has been recommended to enhance lipid accumulation and economic biofuel production in wastewater [90].

A combined process culturing *Chlorella vulgaris* on wastewater centrate (effluent generated through dewatering activated sludge, which derived from secondary wastewater treatment) with the addition of waste glycerol (deriving from biofuel production) resulted in significant removal of N and P and in biomass with 27% of total lipids [91]. Strains of *Chlorella vulgaris* CICESE and *Chlorella vulgaris* UTEX were further able to remove all available N and P from municipal wastewater and produced biomass with a lipid content of 20.5–25.7% per dry weight after 21 days of cultivation [92].

To enhance lipid accumulation in the biomass, the most common approach is exposing the algae to nutrient starvation under phototrophic conditions [90]. Nitrogen starvation is easy to achieve in open-pond cultures and will lead to lipid accumulation; it is dependent on the biomass concentration at the start of starvation, the light availability, and the physiological state of the culture [93]. Wu and coworkers [94] demonstrated that industrial textile wastewater could be used for growth and lipid accumulation of *Chlorella* sp. with chemical oxygen demand (COD) removal efficiency of 75%. The algal biomass contained 20% of fatty acids upon 7 days of cultivation in textile wastewater supplemented with K_2HPO_4 and urea at pH 10 with aeration. Álvarez-Diaz and coworkers [95] selected strains of *Chlorella vulgaris*, *Chlorella kessleri* and *Scenedesmus obliquus* based on their ability to produce lipids when cultivated in municipal wastewater after secondary treatment. *Scenedesmus obliquus* produced a biomass concentration of 1.4 g L^{-1} with lipid content of 37% and a lipid productivity of 29.8 mg L^{-1} d^{-1}, while *C. vulgaris* reached the highest biomass productivity (0.107 g L^{-1} d^{-1}) and a lipid content of 17.23%, corresponding to a lipid productivity of 0.184 g L^{-1} d^{-1}.

The lipid quality of two *Botryococcus braunii* strains grown in municipal wastewater was tested before and after treatment in the aeration tanks [96]. After 10 days of cultivation, the lipid content in pretreated and treated effluent was 24% and 18%, respectively, with N removal efficiencies of 63% and 62% for *B. braunii* LB572 and 61% and 65% for *B. braunii* IBL C116, respectively. The removal of P was 100% in all experiments. According to the authors, *Botryococcus* is a potential lipid source for high-quality biofuel production due to its predominance of saturated fatty-acids [96].

Shen and coworkers [83] studied 20 microalgal strains isolated from a municipal wastewater treatment plant for their ability to treat wastewater and accumulate lipids in their biomass. One strain of *Botryococcus* sp. NJD-1 was selected as the best candidate strain for biofuel production (up to 61.7% lipid content) and removal of pollutants (up to 64.5%, 89.8% and 67.9% for N, P and total organic carbon [TOC]) in pristine wastewater.

3.6. Microalgae Used in Wastewater and Flue-Gas Treatment of Different Origin

The removal of N and P is the primary objective of wastewater treatment by algae. Secondary objectives are the removal of different bioactive contaminants and xenobiotics from wastewater. Treated wastewater has to be separated from the suspended culture and can be released if it complies with the regulations. As mentioned above, wastewater-treatment processes are usually oriented to the production of microalgae biomass or lipid accumulation for biofuel production.

Microalgae species, mainly those of the genus *Chlorella* and *Scenedesmus*, have been shown to be very efficient at removing N and P [97–101]. Nutrient-removal capacities of microalgae have also been studied in *Spirulina* sp., *Nannochlorosis* sp., *Botryococcus brauinii* and *Phormodium bohneri* [86,102–104].

Most studies dealing with microalgal wastewater treatment are performed using municipal wastewater. Laboratory-scale batch cultures of *Chlorella vulgaris* grown in primary treated municipal wastewater removed, after 10 days, over 90% NH_4^+ and 80% PO_4^{3-} [97].

Scenedesmus sp. cultured in non-treated municipal wastewater was reported to remove 85% of NH_4^+ and between 72–76% of PO_4^{3-} in 24 h, while phosphate-starved cells were able to remove 100% NH_4^+ and 87% PO_4^{3-}, in the same period. Thus, PO_4^{3-} starvation as pre-treatment seems to enhance the rate of nutrient removal [105].

Scenedesmus obliquus cultured in cylindrical bioreactors with secondary treated municipal wastewater provided between 47–98% P removal, and 79–100% NH_4^+ removal after 8 days with an estimated biomass productivity of 26 mg^{-1} L^{-1} day^{-1} [98]. A native species of *Scenedesmus* cultured in analogous conditions removed about 99.9% PO_4^{3-} and NH_4^+ daily, with an estimated biomass productivity of 250 mg^{-1} L^{-1} day^{-1}, and 6% total lipids per dry weight after 10 days [100].

C. vulgaris and *S. obliquus* cultivated in secondary treated sewage as free or immobilized cells in bioreactors were reported to remove 60% and 80% NH_4^+ as free cells, and 97% and 100% for immobilized cells; at the same time, PO_4^{3-} removal was 53% and 80% for free cells, and 55% and 83% for immobilized cells, respectively, after only 48 h of treatment [99]. The lipid content of immobilized cells of *C. vulgaris* and *S. obliquus* was 17% and 11%, respectively [99].

A novel approach to treating municipal wastewater was reported [82]; *Scenedesmus dimorphus* was cultivated in floating offshore photobioreactors; the cultures became mixed containing *Chlorella*, *Cryptomonas* and *Scenedesmus* after one year of operation. The authors reported the average lipid content of the algal biomass to be 13%, with a maximum biomass productivity of 22.7 g m^{-2} day^{-1} during a 14-day period [82].

Coelastrum cf. *pseudomicroporum* was cultivated in open ponds filled with municipal wastewater [106]. The obtained biomass was used for carotenoid production and also as a bioflocculating agent to separate non-flocculating green algae (*Scenedesmus ellipsoideus*) and cyanobacteria (*Spirulina platensis*). *C.* cf. *pseudomicroporum* produced up to 91.2 pg $cell^{-1}$ of total carotenoids. The authors confirmed the bioflocculating ability of *C.* cf. *pseudomicroporum* and suggested the alga be used as a bioflocculation tool.

Agricultural wastewater is usually diluted before being used for microalgal cultivation to reduce the high concentrations of nutrients and to decrease turbidity, which restricts light from penetrating the culture [103,107–110]. *Botryococcus brauinii* grown on secondary treated piggery wastewater had an NO_3^- removal efficiency of 98% and an estimated biomass productivity of 700 mg^{-1} L^{-1} day^{-1}, after 12 days of culture [103]. *Botryococcus braunii* cultured in a mixture of cattle feedlot, a hog farm, and a dairy barn, on average removed 88% of total N and 98% of total P in 14 days. After 30 days of growth, it reached an estimated biomass of 84.8 mg^{-1} L^{-1} day^{-1} with total lipids per dry weight of 19.8% [108].

Chlorella pyrenoidosa cultured in the influent and effluent of dairy wastewater reduced the concentrations of NO_3^- and PO_4^{3-} by 60% and 87% in the influent, 49% and 83% in the effluent, respectively, after 10 days of microalgal growth. The estimated biomass productivity and total lipids per dry weight were 6.8 g L^{-1} and 3.5 g L^{-1} respectively in the influent after 15 days of growth [109].

The treatment of anaerobic sludge from a starch wastewater plant with *Chlorella* sp. resulted in 1.67 g L^{-1} and 1.43 g L^{-1} of biomass concentration in heat-treated and untreated anaerobic sludge, respectively, after three days of cultivation [111]. Reported removal efficiencies were 98.3% and 83.0% for N and 64.2% and 89.5% for P, in heat-treated and untreated anaerobic sludge, respectively, after two days of cultivation. Co-cultivation of *Chlorella* sp. with the bacteria *Acinetobacter* sp., which naturally occurs in wastewater, could improve the nutrient-removal efficiencies from centrate wastewater [112]. The observed synergism between the algae and *Acinetobacter* sp. was attributed to the exchange of CO_2 and oxygen.

A microalgal consortium dominated by *Coelastrella* sp. was used for the long-term treatment (1 year) of anaerobically digested wastewater in 9600 L high-rate algae ponds with added CO_2, and resulted in a maximal areal productivity of 29.3 g m^{-2} d^{-1} during the summer period [81].

The microalga *Scenedesmus* sp. was successfully cultivated in starch-containing textile wastewater with a heavy burden of organic compounds. A combined process of activated carbon adsorption, anaerobic digestion and *Scenedesmus* sp. cultivation removed the color by 92.4%, the COD by 90% and even carbohydrates by 98% with high utilization efficiencies of the culture for acetate (95%), propionate (97%) and butyrate (98%) [113]. It was further recently demonstrated that raw tannery wastewater from the leather industry can be used as a source of nutrients by microalgae. Under optimal conditions *Scenedesmus* sp. was able to remove 85% of N, 97% of P and 80% of COD and reached a concentration of 0.90 g L^{-1} of biomass after 24 days of cultivation [114].

The microalga *Spongiochloris* sp. was used to remove hydrocarbons from wastewater originating in the petrochemical industry, which contains mainly diesel hydrocarbons [115]. The alga was reported to produce a maximal biomass of 1.5 g L^{-1} day with 99% removal of total hydrocarbons within 120 days using an airlift photobioreactor [115].

Even marine algae species can be successfully cultivated in wastewater/seawater mixtures; *Nannochloropsis oculata* and *Tetraselmis suecica*, for example, reached biomass concentrations of 1.285 g L^{-1} and 1.055 g L^{-1}, respectively, after 14 days of cultivation in 75% wastewater [116].

During autotrophic growth, microalgae are able to assimilate CO_2 from the atmosphere, flue gases, aqueous CO_2 and soluble carbonates, and convert it into complex molecules [117]. Growth on atmospheric CO_2 is the most basic method; however, its potential yield is limited by the low CO_2 concentration in the air (0.03–0.06% CO_2) and by the low efficiency of CO_2 at diffusing from the atmosphere into the water-based culture [36]. Thus, maximal microalgal growth relies on enhancing

CO_2 concentrations in the culture, either in pure form, compressed or uncompressed, or as part of flue gas. The supplementation of cultures with CO_2 in the form of inorganic carbon, i.e., bicarbonate salts, or as compressed CO_2 gas, enhances photosynthesis and biomass production by up to 256% and 260%, respectively; typically, even a positive effect on lipid production can be observed [118]. Considering microalgal CO_2 uptake ranges from 20% and 70%, at least 1.3–2.4 kg CO_2 are required per kg of dry microalgae [119]; due to the high costs of CO_2 supplementation, bubbling with flue gases provides the best alternative and simultaneously decreases the emission of GHGs [64] and mitigates the costs of chemical and physical treatments of the flue gases [36]. In addition to serving as a source of CO_2, bubbling with flue gases also provides internal mixing of the culture, avoiding concentration gradients of nutrients and allowing all cells to be exposed equally to the light source, so that self-shading and photo-toxicity are minimized. Bubbling of flue gas further controls the pH of a culture, an important factor to consider as a pH-value below eight prevents NH_4^+ to evaporate and PO_4^{3-} to precipitate [57]. Furthermore, this allows the removal of accumulated dissolved oxygen, which is toxic to microalgae [120]. However, to directly inject flue gases into microalgal cultures, a suitable design and operation of the carbonation culture system unit is crucial, otherwise almost all of the CO_2 would be released into the atmosphere [121].

Knowledge of the composition of the flue gases is important and should be monitored as they vary depending on the source of generation, the combustion system and combustion temperature [122]. Although in some flue gases the concentrations of certain compounds, e.g., NO_x, SO_x or heavy metals, are negligible, in others the concentrations exceed toxic limits, causing acidification of the medium or environmental stress to the microalgae [123]. The concentrations of nitrogen oxide for example vary between 50 mg Nm^{-3} and 1500 mg Nm^{-3}, of sulfur dioxide from 0–1400 mg Nm^{-3} and of sulfur trioxide from 0–32 mg Nm^{-3} [122,123]. According to recent reports, the amounts of SO_x and NO_x in the flue gases have to be low to moderate (about 150 ppm) for successful algal cultivation [36,124].

A continuous supply of flue gas into microalgae cultures has also been shown to inhibit the growth of the cells due to the presence of SO_x, NO_x, and a high concentration of CO_2, which causes the pH to decrease. Algae farmers should balance the negative effects of high CO_2 concentrations (decrease of pH affects the utilization of CO_2, then more energy is used from the algae to maintain the intercellular environment pH) with the positive ones (improve photosynthesis and microalgal growth) [125]. The isolation of microalgae strains tolerant to SO_x and NO_x or high CO_2 concentrations, addition of $CaCO_3$ or NaOH to control the culture pH, and applying an on–off pulse for flue gas are some of the strategies tested to overcome these issues [126,127].

Optimal CO_2 concentrations vary greatly among microalgae; most species have been shown to prefer concentrations between 5% and 20%. However, a few Hawaiian local strains of Rodophytes, Chlorophytes and Cyanophyceae efficiently performed photosynthesis when exposed to CO_2 concentrations up to 100%, as long as the pH was controlled in the medium [128–130].

The additional obstacles in the utilization of flue gases are related to the power requirement due to its low pressure, as well as the requirement for cooling operations due to its high temperature. Increases in temperature (>20 °C) can cause a significant reduction in CO_2 solubility, which eventually leads to declined photosynthetic efficiency [120]. Nevertheless, one of the major bottlenecks for the utilization of flue gas in microalgal cultivation is its composition of heavy metals, which ultimately might end up in the biomass [131]. Heavy metals from flue gases have been reported to accumulate in microalgal biomass and to affect both biomass and lipid productivity [131–133]. Heavy metals are potentially toxic to microalgae, and their accumulation in the biomass might further cause carcinogenic, teratogenic and mutagenic effects on human health [134]. The adsorption or uptake of heavy metals by microalgae, therefore, has the potential to limit the use of the biomass [132].

The potential of CO_2 fixation from flue gases combined with wastewater treatment by microalgae has been investigated by a few researchers, and has emerged as a feasible alternative. Biomass and lipid productivity for *Scenedesmus* sp. cultivated with ambient air containing 10% CO_2 were 217.50 and 20.65 mg L^{-1} d^{-1} (9% of biomass), while those for *Botryococcus braunii* were 26.55 and

5.51 mg L^{-1} d^{-1} (21% of biomass), respectively. With flue gas containing 5.5% CO_2, the lipid productivity for *Scenedesmus* sp. and *B. braunii* was increased 1.9-fold (39.44 mg L^{-1} d^{-1}) and 3.7-fold (20.65 mg L^{-1} d^{-1}), respectively [135].

Biomass productivity of *Chlorella* sp. and *Scenedesmus* sp. isolated from a coal-fired thermoelectric power plant was high in the presence of air supplemented with 6% and 12% CO_2, with maximum biomass productivities of 0.087 and 0.085 g dry weight L^{-1} d^{-1}, respectively. Furthermore, these two species were still able to grow in a medium containing up to 18% CO_2 [136]. *C. vulgaris* cultured in industrial wastewater and flue gases from a steel-making plant was able to perform CO_2 fixation and NH_4^+ removal rates of 26 g m^{-3} h^{-1} and 0.92 g m^{-3} h^{-1}, respectively [137].

A mixed culture of *Scenedesmus* sp., *Chlorella* sp., *Nitzschia* sp., *Chlamydomonas* sp., *Oocystis* sp. and *Protoderma* sp. cultivated in agriculture wastewater, i.e., diluted swine manure, with flue gas (7% CO_2) had NH_4^+ removal efficiency of 98% [107].

Three strains from the genus *Scenedesmus* cultivated in a combination of municipal, agricultural (dairy) and industrial (paper and pulp) wastewater in different quantities incorporated with flue gas containing 10% CO_2 demonstrated N and P removals up to 99%, biomass concentrations between 0.62 g L^{-1} and 1.49 g L^{-1}, and lipid yields up to 37% of the dry weight [101]. *Scenedesmus obliquus* grown under mixotrophic conditions in secondary-treated municipal wastewater and agricultural wastewater in different dilutions with flue gases (10–14% CO_2), demonstrated nutrient removal, biomass growth, lipid productivity and carbohydrate productivity up to 22 mg total N L^{-1}, 0.44 g L^{-1}, 11 and 16 mg L^{-1} day^{-1}, respectively, after 6 days of cultivation [124]. *Scenedesmus* sp. cultured in domestic wastewater had a 46.1% increase in biomass productivity with a total of 35.6% lipid and 10.4% carbohydrate upon cultivation in a closed system with the use of 2.5% flue gas CO_2. It also had nutrient-removal efficiencies of about 95% NH_4^+, 66% NO_3^- and 72% PO_4^{3-} [138].

3.7. Removal of Pharmaceuticals

Primary and secondary wastewater treatment have a limited capacity to remove pharmaceuticals from municipal wastewater, because most of these compounds are not metabolized by microorganisms [139]. Wastewater, therefore, contains not only nutrients, but also contaminants like pharmaceuticals or heavy metals. Drugs most frequently found in wastewater are antibiotics, steroids, antidepressants, analgesics, anti-inflammatory, anti-pyretics, beta-blockers, lipid-lowering drugs, sedatives, and stimulants [139]. The concentrations of pharmaceuticals in wastewater, however, are much lower than the reported IC50 values for microorganisms, so that a significant reduction of the algal culture should not occur in tertiary wastewater treatment. The ability of algae to assimilate many common pharmaceuticals prevents their accumulation in natural water bodies.

The microalgae *Chlorella* sp. and *Nitzschia acicularis* were used to remove an endocrine-disrupting compound added to wastewater. The algae were either allowed to swim freely in the wastewater or they were immobilized in alginate. After 10 days of the experiment, the removal rates were 64% and 89% for nitrogen and 90% and 96% for P in the free and immobilized algae, respectively. Furthermore, the authors reported kinetic removal rates of the endocrine-disrupting compounds in the range of 0.01–0.34 d^{-1}, with better removal in the cultures containing alginate beads than in free-swimming microalgae [140].

Chlorella sp., *Chlamydomonas* sp. and *Mychonastes* sp. were used for wastewater reclamation, lipid accumulation and the removal of the antibioticum cephalosporin (100 ppm) [141]. While the lipid production was not inhibited in the algae, the maximum biomass concentration was about 10% lower than in antibiotic free cultures. Cephalosporin disrupts the synthesis of the peptidoglycan layer of bacterial cell walls, but obviously only exhibits weak toxic effects on microalgal growth.

The toxicity of ibuprofen, ciprofloxacin and chlorophenols was tested on microalgae like *Chlorella vulgaris* CCAP 211/11B [142], and IC50 values in the range of 11–90 ppm were determined for these pharmaceuticals. Actual concentration of these pharmaceuticals in wastewater are far lower, and usually they are determined in the ng L^{-1} range [139]. The antibiotics trimethoprim, sulfamethoxazole and triclosan were tested at concentrations of 10 μg L^{-1}, reflecting maximal concentrations detected in wastewater, but no toxic effects were observed on the growth of *Nannochloris* sp. during seven days of incubation [143].

S. obliquus and *Chlorella pyrenoidosa* have been shown to degrade the hormones norgestrel (100% and 60% reduction, respectively) and progesterone (>95% reduction) [144]. *C. pyrenoidosa* was further reported to remove 50% of the biocide triclosan at 800 mg L^{-1} h^{-1} [145].

Chlorella sorokiniana was reported to remove the pharmaceuticals salicylic acid and paracetamol (93% and 42%, respectively) [146]. A mixed population of microalgae species dominated by *Dictyosphaerium* sp. had removal efficiencies of >90% for nine pharmaceuticals, i.e., atenolol, atracurium, bisoprolol, bupropion, citalopram, clarithromycine, diltiazem, metoprolol and terbutaline. Moreover, removal efficiency of 50–90% were registered for an additional 14 pharmaceuticals [147]. The potential of microalgae to degrade other organic pollutants has also been investigated. A mixture of *Chlorella vulgaris* and *Coenochloris pyrenoidosa* degraded p-chlorophenol at a rate of 10 mg L^{-1} day^{-1} [148]. *Scenedesmus obliquus* is able to degrade phenol and dichlorophenols [149].

3.8. Heavy-Metal Contamination in Wastewater

Industrial wastewater often contains heavy metals and organic chemical toxins such as hydrocarbons, biocides and surfactants. Effluents from textile, leather, tannery, electroplating and other metal-processing industries have substantial amounts of toxic metal ions. Often, large industries have their own treatment plants, and therefore these contaminants remain contained in the industrial facilities and do not contribute to municipal wastewater [84].

Contaminants present in the wastewater, e.g., pharmaceuticals or heavy metals, can be taken up by the algae and can accumulate in the biomass, since the algal cell walls are porous and allow the free passage of molecules and ions in aqueous solution, and heavy metals can bind to ligands present in the cell walls. Both living and non-living microalgal cells have been reported as being able to sequester significant quantities of toxic heavy metal ions from aqueous solutions [150,151]. However, the use of non-living cells is more convenient and practical because living biomass cells usually require the addition of growth media which increases BOD or COD in the wastewater effluent [152]. Additionally, non-living algae is not affected by the toxicity of the heavy metals. Thus, non-living biomass can be subjected to chemical and physical treatment techniques to improve its performance, and adsorbed metals can be easily recovered from the biomass leading to repeated use of the biomass, and consequently a more profitable process.

Living microalgae have been used to perform quaternary wastewater treatment, which is designed to remove organics, heavy metals, and soluble minerals [153]. The sequestering mechanism employed depends on the structure and concentration of chemical substances, temperature, pH, and nutrient accessibility in the cultivation medium, along with cell size, number, the eco-physiological capacities of the applied species and their cell conditions (living or non-living) [154]. Some examples of sequestering processes are physical absorption, ion exchange, chemisorption, covalent bonding, surface precipitation, redox reactions and crystallization in the cell surface [155]. Biochemical transport mechanisms across the algal cell membranes include passive diffusion, facilitated diffusion, or active transport, with different transport rates [156]. Metals are taken up either by biosorption (passive uptake), where the metal ion binds to the cell wall, or by ATP-dependent active uptake through the membrane [157]; the choice of transport usually is dose dependent [158]. At low metal ion concentrations, active transport is favored by the cell [156]; trace concentrations of heavy metals in municipal wastewater usually do not inhibit significantly the growth of the algal consortium. Many algae strains even show high tolerance to trace amounts of Hg, Cd, Pb and Cr [58,159–162].

In order to eliminate the negative impacts of pollutants in wastewater on microalgal growth, Chang et al. proposed an annular ion-exchange-membrane photobioreactor [163]. Direct mixing of the algae cells with wastewater is excluded in this bioreactor by separating the culture from wastewater in two different chambers, so that only nutrients are exchanged. The growth of *C. vulgaris* has been tested in this bioreactor on synthetic wastewater medium and also on untreated tannery industrial effluent with a high Cr concentration. The authors reported a biomass concentration of 2.04 g L^{-1} after 12 days of the experiment, with removal efficiencies of 87% for N, 71% for P and 89% for Cr.

4. The Use of Algal Biomass

The recycling of nutrients in wastewater reclamation is crucial for achieving environmental sustainability. The production of N and P fertilizers has a high energy requirement, 11.1 kWh kg^{-1} and 10 kWh kg^{-1}, respectively [11]; hence, recycling N and P will not only be sustainable, but even save a huge amount of energy at the global scale. As described above, algae are highly efficient in removing pollutants such as heavy metals, pharmaceuticals and emerging contaminants, which is important for wastewater reclamation; however, as pointed out, the aforementioned contaminants will accumulate in the algal biomass. Hence, biomass received from algae grown in wastewater and certain flue gases cannot be used as food, feed or even as biofertilizers. Consequently, algae grown on polluted wastewater and flue gases should be used as a source of bioenergy (Figure 1), where the contaminants can be concentrated in ashes or separated after extraction or transformation of energy-rich compounds.

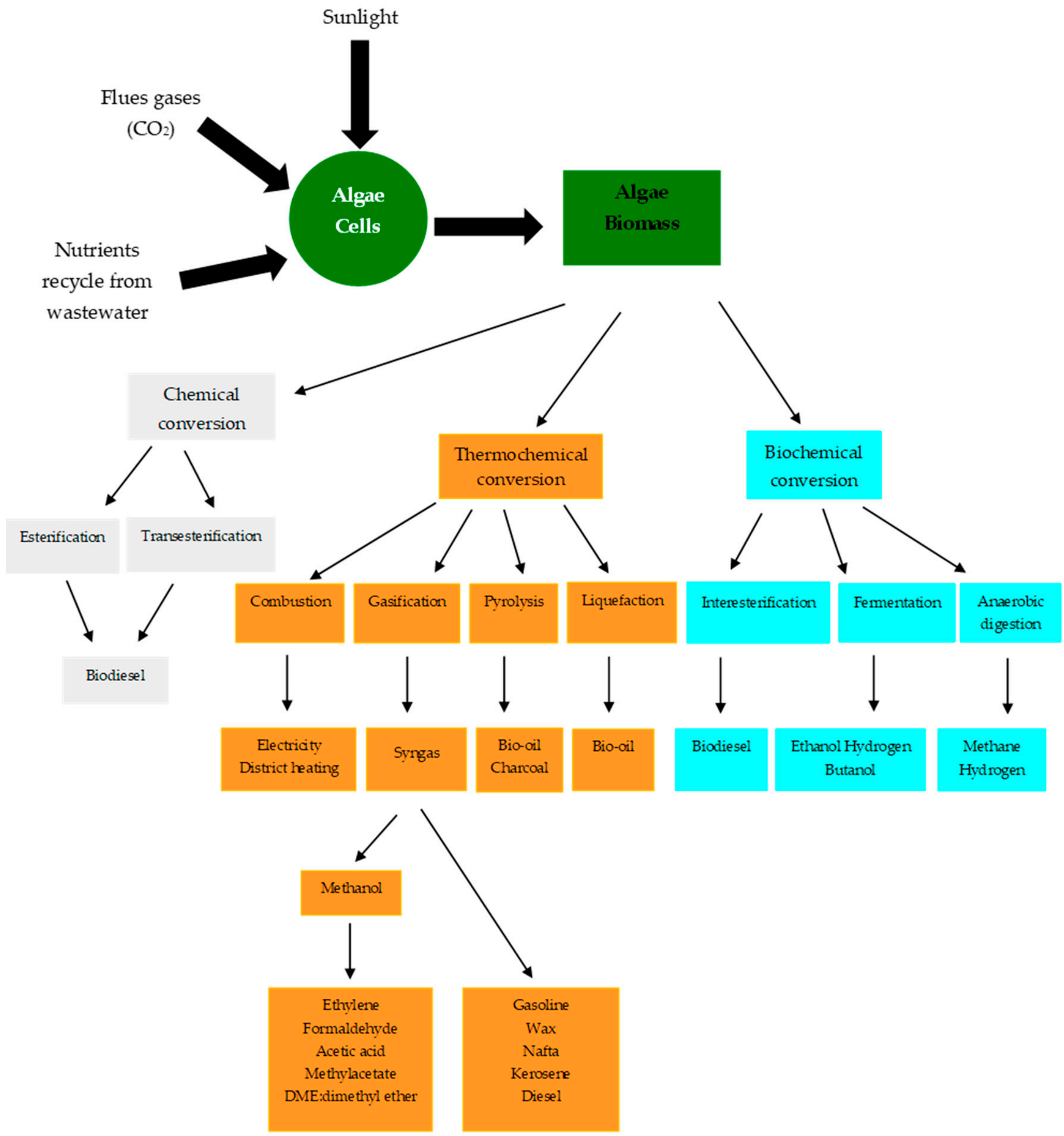

Figure 1. Algal biomass conversion pathways (modified from [84]).

Even though biodiesel derived from microalgal biomass is of high potential, there are several challenging technical barriers that need to be solved before making this algal biofuel a reality. These challenges include the development of cost- and energy-effective cultivation and harvesting techniques, sustainable and energy-efficient lipid-conversion technology [164].

4.1. Chemical Conversion of Algal Lipids

Lipids are extracted from the algal biomass and chemically treated to produce fatty acid methyl esters (FAMEs), or fatty acid ethyl esters (FAEEs) biodiesel. The chemical conversion includes esterification and transesterification (Figure 1). Even though esterification and transesterification are well-known chemical conversion techniques at the industrial scale for FAME production from vegetable oils, when applied to algae an extra production step has to be included to disrupt the tough cell wall surrounding many algae species.

4.1.1. Esterification

The esterification process is a reversible reaction, where an acid reacts with an alcohol forming an ester. Esterification can be performed by the addition of a homogeneous or heterogeneous catalyst [165]. Homogeneous acid (HCl, H_2SO_4) and alkaline catalysts (NaOH, KOH) are the most traditional, while heterogeneous solid acid catalysts are good alternatives [165], even though their results vary considerably among algal species [166]. The heterogeneous solid catalyst Amberlyt-15 showed a free fatty acid (FFA) conversion of 84% in *Chlorella protothecoides* and 35% in *Scenedesmus obliquus*. The difference in converting the FFA of the two algal strains was found to be caused by their higher molecular weight in *Scenedesmus obliquus*, which induced shear stress; the addition of 94% H_2SO_4 resulted in the highest FFA conversion [165]. Simultaneous esterification and transesterification reactions using acid catalysts reduce saponification, resulting in an almost complete conversion into biodiesel [84].

4.1.2. Transesterification

Biodiesel is a substitute fuel, manufactured from renewable resources and waste oils. Biodiesel production requires relatively simple chemical reactions; most common is transesterification, and mainly alkali-catalyzed transesterification: a triglyceride (TAG) reacts with an alcohol (most frequently ethanol or methanol) in the presence of a catalyst [25,84]. The conversion of the TAG to FAME or FAEE yields glycerol as a byproduct. As mentioned above for esterification, catalysts can be homogeneous (same phase as reactant) or heterogeneous (different phase, usually solid); furthermore, catalysts used for biodiesel production fall into three categories: alkalines, acids, and enzymes. Most frequently, alkaline catalysts are used for the transesterification of fatty acids; however, algae often contain large quantities of FFAs providing a negative impact on alkaline catalysts. Hence, acid catalysts are mainly used to transesterify algal lipids.

Even though algal biomass can contain a high ratio of lipids, only a portion of the total lipids are FFAs and TAGs producing FAMEs (biodiesel). Consequently, the FAMEs yield as a percentage of total lipid and dry algal biomass can vary greatly, ranging from 0.6% to 33.5% of dry weight [167]. Biodiesel has been shown previously to contain superior lubricity to conventional diesel [168]. However, biodiesel also emits increased amounts of NO_x when used as a pure or blended fuel in two- and four-stroke engines [168]. Using a B20 blend, with the biodiesel portion containing 76% methyl oleate, the NO_x emissions could be eliminated and a NO_x neutral blend produced [169]. Studying algae wastewater treatment with the addition of CO_2, the green algae *Chlorella vulgaris* was shown to contain an oleic acid concentration of 44% after 7 days of cultivation [170] leading to biodiesel of good quality with low NO_x emissions.

4.2. Thermochemical Conversion

A very interesting process for producing biofuels is the conversion of the entire harvested algal biomass to fuel using a thermochemical process (Figure 1) [171–174]. Thermochemical conversion allows the use of the entire algal biomass and not just the lipids or carbohydrates. Special cultivation conditions, such as nutrient stress to increase the lipid content, are not needed. The thermochemical process offers even the possibility to inactivate or destroy certain pollutants present in the biomass, i.e., pharmaceuticals or micropollutants. Combustion is the best-known and most widely used thermochemical conversion process; however, algae have a very high content of intracellular water, even after the removal of extracellular water and, therefore, are not suitable for direct combustion without a drastic reduction of the intracellular water content. Other thermochemical processes, which apply a high temperature to dry the biomass in the partial or complete absence of oxygen, might be better suited from a sustainability perspective. These processes, including gasification, hydrothermal liquefaction and pyrolysis [171,173], transform the biomass into a mixture of solids, liquids and gases.

4.2.1. Combustion

Combustion is a thermochemical process used to produce electricity and district heating. The combustion of biomass is feasible only when it contains moisture of less than 50%. Biomass is usually burnt in the presence of air in a furnace, boiler, or steam turbine at temperatures above 800 °C [117]. Algal biomass grown on artificial substrates has been shown to contain a high ash concentration, ranging from 7.9–37.8% of the dry weight with a calorific value of 15.4 MJ kg^{-1} and 19.05 MJ kg^{-1}, respectively [175]. Oxygen concentrations ranging from 25–35 vol % were shown to be optimal for the combustion of *Chlorella vulgaris* biomass [176]. Compared to coal, algal biomass usually contains higher amounts of ash, moisture, reactivity and volatile matter. Concentrations of the following ions are higher in algae ash than in ash originating from coal: Ag-, As-, Au-, B-, Be-, Br-, Ca-, Cd-, Cl-, I-, K-, Mg-, N-, Na-, O-, P-, Si-, Sn-, Sr- and Zn. However, the ash-fusion temperatures required are lower for algal biomass than for coal, including initial ignition temperature, combustion temperature, bulk density, fixed carbon, and heating value [177]. A lifecycle analysis study dealing with the environmental impact of wastewater-based algae biofuels showed that, among 16 different pathways, only one had an environmental footprint lower than petroleum diesel when the algae were cultivated on municipal centrate and the biomass was processed by wet lipid extraction or combustion [178]. An advantage of using combustion is its use of the entire algal biomass; additional extractions or fractions are not needed. Furthermore, combustion destroys several pollutants such as pharmaceuticals and other emerging contaminants accumulating in algal biomass produced in wastewater. We believe that combustion of algal biomass is a good solution to produce energy from algae grown on wastewater, as long as their biomass is dried efficiently using solar energy or waste heat (i.e., industrial).

4.2.2. Pyrolysis

Pyrolysis is a thermochemical conversion of dry biomass that occurs in the absence of oxygen at temperatures below 550 °C [179,180]. There are different types of pyrolysis such as slow, fast and catalytic pyrolysis [181]. Pyrolysed biomass produces a mixture of different fractions such as biochar, bio-oil and gases (methane, hydrogen, carbon monoxide and carbon dioxide) [117,174,179]. Their relative abundance is dependent on the temperature used, and hence additional separation steps and upgrading reactions are required after the process. Recently, 50 ± 2 wt.% of the initial algal biomass grown on wastewater was pyrolysed at 500 °C; the elemental analysis of the resulting bio-oil contained >65 wt.% C, 6–9 wt.% N, 8–10.2 wt.% H with an energy content of 34.4–37 MJ kg^{-1} [179]. Pyrolysis of biomass from *Scenedesmus obliquus* grown on brewery wastewater resulted in a bio-oil yield of 64% on a dry weight basis [182]. Different algal species pyrolyse differently, as indicated by the variation of the composition of the liquid, gas and char fractions [183]. In a recent study it was suggested, from an energy perspective, using non-condensable gases and char as fuel in the process to make algal-based bio-oil feasible [179].

While pyrolysis offers relatively low capital costs and relatively high biofuel yields, its drawback is the rather poor fuel quality [174].

4.2.3. Liquefaction

Wet biomass can be converted in a process called hydrothermal liquefaction (HTL) with hot compressed water functioning as a reaction medium. Compared to pyrolysis, HTL is performed at lower temperatures ranging from 200–375 °C, but higher pressures in the range 4–40 MPa [184,185] resulting in higher capital investment. In HTL, catalysts are utilized to accelerate the reaction; recovering the catalyst is often challenging as it inactivates over time. Temperature, total solids, algal lipid content, ash content and retention time are factors that affect bio-oil yields and quality [174,186,187].

The HTL liquid fraction, a complex mixture of different organic compounds, is often called bio-oil or biocrude. Depending on the feedstock and the process used, the bio-oil may need additional fractionation and upgrading to produce a good quality fuel. Fractionation of complex bio-crude yields mixtures of gasoline, diesel and heavy oil, in ratios of e.g., 25%, 50%, and 25%, respectively [188].

One of the major challenges of fuels produced by HTL from algal biomass is their high N, oxygen and sulfur content. If left in the fuel, high levels of NO_x will be produced when N is combusted; as a consequence, it first must be removed during the upgrading process [174]. A recent study investigated the processing of two algal species by HTL at different temperatures: *Galdieria sulphuraria* has high protein content and is of interest for wastewater treatment, while the marine algae *Nannochloropsis salina* is rich in lipids. It was concluded that carbohydrates have to be removed from the biomass prior to HTL, while N can be removed during the catalytic upgrading [189]. A two-stage HTL was proposed, which would allow the reduction of the N content in the biocrude of more than 50% [190]. A high ash content in the algal feedstock was shown to affect neither the high heating value, nor the boiling point distribution of the algal bio-crude oil. High-ash algal biomass from wastewater treatment systems, therefore, is suitable for HTL without requiring multi-step pretreatments or modifications for biofuel application [187].

In conclusion, the main advantage of HTL is the use of wet algal biomass (algae slurry), while its main drawback is the relatively high operational cost. We should bear in mind that algal cultures in wastewater consist of polycultures varying from season to season; a homogeneous biomass feedstock, which often is demanded by an industrial-conversion processes, therefore cannot guaranteed.

4.2.4. Gasification

Biomass gasification is a thermochemical conversion process occurring at temperatures above 375 °C and pressure above 20 MPa under a controlled amount of oxygen. The limited amount of oxygen leads to an incomplete combustion of the biomass, resulting in the production of combustible gases like CO, H_2, and low molecular weight hydrocarbon gases [174,191,192]. There is a great variation in the yield and the composition of the different gases produced by algal biomass gasification [193]. Gasification can be performed on wet (hydrothermal gasification) or dry biomass. Syngas can also be used as a feedstock in the production of chemicals (e.g., methanol; [194]). The gasification of biomass derived from the algae *Acutodesmus obliquus* with dry matter content of 5% resulted in gasification efficiencies of up to 98% when gasified at a temperature of 600 °C [195].

A novel energy-production system was proposed using nitrogen cycling combined with low-temperature catalytic gasification of the microalgal biomass. In such a process, biomass containing high moisture can be gasified directly into methane-rich fuel gas without including a drying step; at the same time, nitrogen present in the biomass is converted to NH_4^+. It has been claimed that the integration of biological- (CO_2-fixation by algal photosynthesis) and thermal-based conversion technologies in a farm-scale hybrid design can recover energy via wet gasification, drastically reduce CO_2 emissions, and efficiently recycle nutrients. According to Cantrell and coworkers, such a design would potentially make large-scale intensive animal farming operations sustainable and environmentally friendly by generating on-farm renewable energy [191].

Gasification has the advantage of allowing the use of wet algal biomass. The downside of gasifying algae biomass is the possible formation of agglomerates in the gasifier, and operational modifications therefore have to be applied.

4.3. Biochemical Conversion

4.3.1. Interesterification

Interesterification can be performed chemically or enzymatically and leads to a rearrangement of fatty acids in TAGs. The TAG structure is transformed by the reaction of the fatty acids esters with other fatty acids, alcohols or esters [196]. Recently, interest has grown in enzymatic interesterification processes for biodiesel production from algal oil [84,197]. The reaction of TAGs with ethyl acetate in the presence of the enzyme lipase produces new esters and triacetin without the formation of glycerol as a byproduct; these lipases can be either extracellular or intracellular immobilized [84]. The energy content of the produced biofuel increases due to the formation of triacetin; compared to conventional biodiesel, energy increases of 11% and 2.8% were obtained when the same amount of algal oil was treated with catalytic and enzymatic interesterification, respectively [197].

4.3.2. Anaerobic Digestion

Anaerobic digestion of cyanobacteria, macroalgae or microalgae [198] grown in wastewater [199] is a well-established method. The amount of lipids, carbohydrates and proteins as well as the C/N ratio of the biomass greatly affect the biogas production, often limiting productivity. Ammonia produced during the anaerobic digestion of the algal biomass (during hydrolysis of proteins) was found to inhibit methanogenic bacteria. Originally this ammonia-inhibition was thought to be caused by the increasing pH [199], but lately several other factors have been found to be equally important: imbalance of the (low) C/N ratio [200], nature of the feed and the inoculum; furthermore, other parameters such as pH and the presence of antagonistic ions like Na^+, Ca^{2+}, and Mg^{2+} play an important role [201–204]. High ammonium concentration at alkaline pH converts acetate (a main substrate for the methanogens) into ammonium acetate or ammonium bicarbonate and results in the depletion of available acetate to the methanogens [200,205]. Co-digestions, preferably with substrates that will improve the carbon content, seem to solve this problem [206,207]. Co-digestions of different types of sludge and algal biomass have been performed [208,209]. A statistically significant synergistic effect was observed when 63% (*w*/*w* VS, volatile solids) of undigested sewage sludge was mixed with 37% (*w*/*w* VS) of wet algal slurry; the mixture produced 23% more methane than digested sewage sludge alone [208]. Interestingly, co-digestion of *Chroococcus* biomass with cattle-dung resulted in up to 292 mL CH_4 g^{-1} VS fed with a C/N ratio of 13/1. In contrast, only 202 mL and 142 mL CH_4 g^{-1} VS fed was recorded for algae (C/N 9.26/1) and cattle dung (C/N 31.56/1) alone, respectively [210]. Alternatively, nitrogen-limited biomass has to be used for anaerobic digestion; with this material the production of inhibitory substances is negligible, while extraordinarily high biogas amounts are produced in a stable process. With nitrogen-limited biomass, biogas and the biomass-to-methane energy conversion efficiency was up to 84% [211].

The algal cell wall is an important factor affecting biogas production from algal biomass. The thick cell walls of some algae species limit digestibility during anaerobic digestion [212]; hence pretreatments of the biomass are required for biogas production from microalgae grown in wastewater [213,214].

Thermal, mechanical, chemical and biological pretreatments have been shown to be effective at increasing biomass solubilization and methane yield; unfortunately, these pretreatments are species-dependent [214].

Anaerobic digestion is a well-known and widely used method that has the advantage of using algal slurry without the need for dewatering; however, this method might demand pretreatments to break the cell wall, depending on the algae species to be digested. Another drawback of anaerobic digestion of algae grown on wastewater and flue gases is the relatively high heavy metal content of the digestate that, as a consequence, cannot be used as soil fertilizer.

4.3.3. Fermentation

- Bioethanol

Algal biomass can be used as a raw material for ethanol production [84,215,216]. Many algae store fixed carbon as starch that can be extracted from the harvested algal biomass mechanically or enzymatically and converted to sugars to feed any form of fermentation. The extracted starch is hydrolyzed either chemically or enzymatically. Enzymatic hydrolysis using thermostable enzymes showed a very high recovery of extracted glucose g g^{-1} total sugar and a higher bioethanol yield than acid-hydrolyzed mixed algal biomass [217]. Once the cells start to degrade, yeast (*Saccharomyces cerevisiae*) is added to the biomass to ferment the sugars into ethanol. The enzyme invertase catalyzes the hydrolysis of sucrose to convert it into glucose and fructose; then, a second enzyme, zymase, converts the glucose and the fructose into ethanol [84].

Algae that store carbon as starch can be cultivated under conditions that maximize starch formation, e.g., a *Chlorella* strain grown under sulfur limitation could produce up to about 50% starch by weight [77]. Different algae species vary in their content of carbohydrates, ranging from 4–64% expressed on a dry-matter basis [164]. In *Scenedesmus obliquus*, the amount of glucose increased significantly from 53% to 73% of the total carbohydrates as light intensity rose from 60 to 180 μmol photons m^{-2} s^{-1} [218]. The majority of studies on fermenting algal biomass have been performed with algae cultured in media; however, even microalgae grown in wastewater have been shown to be suitable for the production of bioethanol [219].

- Biobutanol

Biobutanol is a four-carbon alcohol that can be used as a biofuel when blended with gasoline and has the potential to act as biodiesel when blended with diesel [220]. Due to its chemical structure, the energy content of biobutanol is higher than that of bioethanol. Algal biomass can be used to produce biobutanol via bacterial fermentation of carbohydrate-rich biomass [221–223]; the most commonly used are bacteria of the genus *Clostridium* [192,193]. Biobutanol has been produced from algae grown in wastewater, e.g., *Neochloris aquatic* grown in swine wastewater [221,222]. Fermentation of the carbohydrate-rich fraction of the algal biomass after lipid extraction (algal acid hydrolysates) achieved a butanol yield of 8.05 g L^{-1} [223].

Compared to lower carbon alcohols such as methanol and ethanol, biobutanol has the advantage of higher heating value and causes fewer ignition problems. It is easier to blend with traditional fuels, safer and less corrosive; furthermore, in spite of its higher viscosity, butanol does not create problems for sensitive fuel pumps [220]. However, one should not neglect the fact that the octane value of butanol is lower compared to lower carbon alcohols. The presence of certain protein components after pretreatment can also inhibit the fermentation process [221].

- Biohydrogen

Biohydrogen has the highest energy content per mass, and can be converted to electricity in a fuel cell. Biohydrogen can be produced by bacteria fermenting algal biomass [224–227]; the production of biohydrogen is also called dark fermentation. Anaerobic solid-state fermentation of *Chlorella* sp. reached a maximal hydrogen yield of 165 ± 12 mL H_2, equivalent to 18.58 mL H_2 g^{-1} VS and 0.28 L H_2 L^{-1} reactor d^{-1} [228]. Wet and dry biomass of *Scenedesmus obliquus* was fermented for the production of biohydrogen using *Enterobacter aerogenes* and *Clostridium butyricum* with similar rates of biohydrogen production and purity independent of the water-state of the biomass; hence, the energy-consuming step of drying the biomass seems not to be necessary [225].

A combination of microalgal urban wastewater treatment with biohydrogen production was investigated using *Chlorella vulgaris*, *Scenedesmus obliquus,* and a consortium of wild algal species. After nutrient-depletion, the microalgae were kept for two more weeks in the photobioreactor to induce sugar accumulation (22–43%); the algae biomass was then fermented by *Enterobacter aerogenes*. The H_2 yield gained from *Scenedesmus obliquus* biomass was similar to that gained from algae grown in synthetic media [224].

Biohydrogen is highly advantageous, containing lower emissions than carbon-based fuels since the combustion of hydrogen releases no carbon dioxide. Nowadays, hydrogen fuels are mainly produced from natural gas, hence the production of biohydrogen via the fermentation of algae grown in wastewater and flue gases is clearly beneficial for the environment with its much lower environmental footprint. The fermentation of algal biomass for the production of biohydrogen is a rather unexplored field; more research is needed considering the organic and inorganic contaminants present in algal biomass produced in wastewater and flue gases; those possibly could inhibit fermentation.

5. Conclusions

Clearly wastewater of different origin such as municipal, industrial and agricultural, offers a great source of nutrients for algal growth. They reduce the operational cost of algal cultivation compared to expensive media containing fertilizers or nutrients at the same time as nutrients are recycled, and energy is saved during wastewater reclamation. The ability of microalgae to grow at different light conditions, various carbon sources, and a broad range of pH and temperatures, makes them ideal to withstand diurnal variation in pH, light intensity, temperature and DO in open ponds. Their desired characteristics include spontaneous auto-flocculation in the late stationary phase for easier biomass collection and a high lipid content, which can be induced by stresses like e.g., nutrient deprivation. Their remarkable adaptability to different nutrient sources and high resistance to numerous contaminants present in wastewater turns them into unique unicellular factories that, driven by photosynthesis, can clean wastewater and produce valuable biomass as well as lipids designated for biofuel synthesis. However, wastewater does not contain only nutrients for algal growth, but also pollutants (pharmaceuticals, heavy metals) that directly inhibit algal growth and later influence the quality and future use of biomass. Similarly, flue gases are a very valuable source of carbon for the photosynthetic algae, but even they contain other chemicals including heavy metals. Hence, microalgal strains used for wastewater and flue-gas treatment have to be robust and to exhibit high growth rates as well as tolerance to biotic (e.g., grazers) and abiotic contaminants like heavy metals and pharmaceuticals.

The biomass and its compounds (lipids, pigments, polyunsaturated fatty acids (PUFA)) of microalgae grown on wastewater is not suitable for use as food or feed. Instead, extractions of the algal biomass can provide lipids for biofuel production, products of higher value, or N and P to be used as biofertilizers. The remaining biomass can be anaerobically digested to produce biogas, fermented or thermochemically converted (Figure 1). A possibility for improving the energy recovery of algal biomass is the combination of different conversion processes, i.e., FAMEs, with anaerobic digestion or fermentation. The industrial sector is seeking a large, homogeneous supply of biofuels and other biomaterials, a need that algae, theoretically, perfectly meets; however, algal biomass when grown on wastewater can vary greatly both in quality and quantity during the growth season. Hence, it is pivotal to characterize the quality of the biomass produced during the entire growth season; furthermore, the conversion of algal biomass into biofuel should be performed by a method suitable for variations in biomass quality, but still producing a biofuel of high quality. Various methods, therefore, require pretreatment or co-digestion with other types of biomass to optimize production. The thermochemical conversion pathways appear particularly interesting for processing algal biomass and should be chosen based on the target biofuel: the production of electricity or district heating combustion is an appealing option, while pyrolysis or HTL are the choice for gaining liquid fuels. In any case, a complete characterization of the algal biomass at the time of harvest or even during the entire growth season is needed in order to facilitate the choice of product and conversion method. We should bear in mind that the producers of algal biomass and the processing biotechnologists have to work hand in hand to be able to fully exploit its rich energy content. Furthermore, the obvious environmental benefits arising from algal cultivation in wastewater and flue gases should not be overlooked especially in places where water is a limiting factor.

In summary, wastewater and flue-gas reclamation can be combined efficiently for the generation of bioenergy from microalgae. However, for the economic realization of this approach, political decisions for a sustainable future are needed. Future studies focusing on pre-treatments of algal biomass are needed to optimize the energetic value "entrapped" in algal cells.

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References

1. United Nations. *World Population Prospects: The 2006 Revision, Highlights*; Working Paper No. Esa/p/wp. 202; Department of Economic and Social Affairs, Population Division, United Nations: New York, NY, USA, 2007.
2. International Energy Agency. *Key World Energy Statistics*; International Energy Agency Publications: Paris, France, 2017.
3. Hill, J.; Nelson, E.; Tilman, D.; Polasky, S.; Tiffany, D. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 11206–11210. [CrossRef] [PubMed]
4. Ormerod, W.G.; Freund, P.; Smith, A.; Davison, J. *Ocean Storage of CO_2*; International Energy Agency Greenhouse Gas R&D Programme: Cheltenham, UK, 2002.
5. *European Council Directive 2009/28/EC on the Promotion of the Use of Energy from Renewable Sources and Amending and Subsequently Repealing Directives 2001/77/EC and 2003/30/EC*; European Council: Brussels, Belgium, 2009.
6. Beurskens, L.W.M.; Hekkenberg, M. *Renewable Energy Projections as Published in the National Renewable Energy Action Plans of the European Member States*; Energy Research Centre of the Netherlands and European Environment Agency: Petten, The Netherlands, 2011.
7. European Commission. *Energy 2020—A Strategy for Competitive, Sustainable and Secure Energy*; European Commission: Brussels, Belgium, 2010.
8. Food and Agricultural Organization (FAO). *Introducing the International Bio-Energy Platform*; FAO: Rome, Italy, 2006.
9. Hay, R.K.M.; Porter, J.R. *The Physiology of Crop Yield*; Blackwell Publishing: Oxford, UK, 2006.
10. Zhang, Q.; Chang, J.; Wang, T.; Xu, Y. Review of biomass pyrolysis oil properties and upgrading research. *Energy Convers. Manag.* **2007**, *48*, 87–92. [CrossRef]
11. Olsson, G. *Water and Energy: Threats and Opportunities*; IWA Publishing: London, UK, 2015.
12. Fargione, J.E.; Plevin, R.J.; Hill, J.D. The ecological impact of biofuels. *Annu. Rev. Ecol. Evol. Syst.* **2010**, *41*, 351–377. [CrossRef]
13. McKendry, P. Energy production from biomass (part 1): Overview of biomass. *Bioresour. Technol.* **2002**, *83*, 37–46. [CrossRef]
14. European Commission. *The Impact of Biofuels on Transport and the Environment, and Their Connection with Agricultural Development in Europe*; Directorate General for the Internal Policies Policy Department B: Structural and Cohesion Policies—Transport and Tourism; European Commission: Brussels, Belgium, 2015.
15. Gibbs, H.K.; Brown, S.; Niles, J.O.; Foley, J.A. Monitoring and estimating tropical forest carbon stocks: Making redd a reality. *Environ. Res. Lett.* **2007**, *2*, 045023. [CrossRef]

16. Fargione, J.; Hill, J.; Tilman, D.; Polasky, S.; Hawthorne, P. Land clearing and the biofuel carbon debt. *Science* **2008**, *319*, 1235–1238. [CrossRef] [PubMed]
17. Larson, E.D. A review of life-cycle analysis studies on liquid biofuel systems for the transport sector. *Energy Sustain. Dev.* **2006**, *10*, 109–126. [CrossRef]
18. Dominguez-Faus, R.; Powers, S.E.; Burken, J.G.; Alvarez, P.J. The water footprint of biofuels: A drink or drive issue? *Environ. Sci. Technol.* **2009**, *43*, 3005–3010. [CrossRef] [PubMed]
19. Immerzeel, D.J.; Verweij, P.A.; van der Hilst, F.; Faaij, A.P.C. Biodiversity impacts of bioenergy crop production: A state-of-the-art review. *GCB Bioenergy* **2014**, *6*, 183–209. [CrossRef]
20. Mitchell, D. *A Note on Rising Food Prices*; Police Research Working Paper; World Bank: Washington, DC, USA, 2008; Volume 4682.
21. Eisberg, N. *Harvesting Energy: Neil Eisberg Catches Up on the Latest Developments in Second-Generation Bioethanol Production.(Biofuels)*; Ten Alps Publishing: London, UK, 2006.
22. Zhang, J.; Osmani, A.; Awudu, I.; Gonela, V. An integrated optimization model for switchgrass-based bioethanol supply chain. *Appl. Energy* **2013**, *102*, 1205–1217. [CrossRef]
23. Banse, M.; Meijl, H.V.; Tabeau, A.; Woltjer, G.; Hellmann, F.; Verburg, P.H. Impact of eu biofuel policies on world agricultural production and land use. *Biomass Bioenergy* **2011**, *35*, 2385–2390. [CrossRef]
24. Secchi, S.; Kurkalova, L.; Gassman, P.W.; Hart, C. *Land Use Change in a Biofuels Hotspot: The Case of Iowa, Usa*; Department of Economics, Iowa State University: Ames, IA, USA, 2011.
25. Chisti, Y. Biodiesel from microalgae. *Biotechnol. Adv.* **2007**, *25*, 294–306. [CrossRef] [PubMed]
26. Li, Y.; Horsman, M.; Wu, N.; Lan, C.Q.; Dubois-Calero, N. Biofuels from microalgae. *Biotechnol. Prog.* **2008**, *24*, 815–820. [CrossRef] [PubMed]
27. Li, Y.; Horsman, M.; Wang, B.; Wu, N.; Lan, C.Q. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. *Appl. Microbiol. Biotechnol.* **2008**, *81*, 629–636. [CrossRef] [PubMed]
28. Metting, F.B. Biodiversity and application of microalgae. *J. Ind. Microbiol.* **1996**, *17*, 477–489. [CrossRef]
29. Schenk, P.M.; Thomas-Hall, S.R.; Stephens, E.; Marx, U.C.; Mussgnug, J.H.; Posten, C.; Kruse, O.; Hankamer, B. Second generation biofuels: High-efficiency microalgae for biodiesel production. *Bioenergy Res.* **2008**, *1*, 20–43. [CrossRef]
30. Griffiths, M.J.; Harrison, S.T.L. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J. Appl. Phycol.* **2009**, *21*, 493–507. [CrossRef]
31. Ullah, K.; Ahmad, M.; Sofia; Sharma, V.K.; Lu, P.; Harvey, A.; Zafar, M.; Sultana, S.; Anyanwu, C.N. Algal biomass as a global source of transport fuels: Overview and development perspectives. *Prog. Nat. Sci. Mater.* **2014**, *24*, 329–339. [CrossRef]
32. Rodolfi, L.; Chini Zittelli, G.; Bassi, N.; Padovani, G.; Biondi, N.; Bonini, G.; Tredici, M.R. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol. Bioeng.* **2009**, *102*, 100–112. [CrossRef] [PubMed]
33. Mata, T.M.; Martins, A.A.; Caetano, N.S. Microalgae for biodiesel production and other applications: A review. *Renew. Sust. Energy Rev.* **2010**, *14*, 217–232. [CrossRef]
34. Delucchi, M. *A Lifecycle Emissions Model (LEM): Lifecycle Emissions from Transportation Fuels, Motor Vehicles, Transportation Modes, Electricity Use, Heating and Cooking Fuels, and Materials*; Institute of Transportation Studies, University of California: Davis, CA, USA, 2003.
35. Pittman, J.K.; Dean, A.P.; Osundeko, O. The potential of sustainable algal biofuel production using wastewater resources. *Bioresour. Technol.* **2011**, *102*, 17–25. [CrossRef] [PubMed]
36. Wang, B.; Li, Y.; Wu, N.; Lan, C.Q. CO_2 bio-mitigation using microalgae. *Appl. Microbiol. Biotechnol.* **2008**, *79*, 707–718. [CrossRef] [PubMed]
37. Lundquist, T.J.; Woertz, I.C.; Quinn, N.; Benemann, J.R. *A Realistic Technology and Engineering Assessment of Algae Biofuel Production*; Energy Biosciences Institute: Berkeley, CA, USA, 2010.
38. Norsker, N.-H.; Barbosa, M.J.; Vermuë, M.H.; Wijffels, R.H. Microalgal production—A close look at the economics. *Biotechnol. Adv.* **2011**, *29*, 24–27. [CrossRef] [PubMed]
39. Quinn, J.C.; Yates, T.; Douglas, N.; Weyer, K.; Butler, J.; Bradley, T.H.; Lammers, P.J. Nannochloropsis production metrics in a scalable outdoor photobioreactor for commercial applications. *Bioresour. Technol.* **2012**, *117*, 164–171. [CrossRef] [PubMed]

40. 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. *Bioresour. Technol.* **2011**, *102*, 159–165. [CrossRef] [PubMed]
41. Oswald, W.J.; Gotaas, H.B. Photosynthesis in sewage treatment. *Trans. Am. Soc. Civ. Eng.* **1957**, *122*, 73–105.
42. Vitousek, P.M.; Aber, J.D.; Howarth, R.W.; Likens, G.E.; Matson, P.A.; Schindler, D.W.; Schlesinger, W.H.; Tilman, D.G. Human alteration of the global nitrogen cycle: Sources and consequences. *Ecol. Appl.* **1997**, *7*, 737–750. [CrossRef]
43. Carpenter, S.R.; Caraco, N.F.; Correll, D.L.; Howarth, R.W.; Sharpley, A.N.; Smith, V.H. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* **1998**, *8*, 559–568. [CrossRef]
44. De la Noüe, J.; Laliberté, G.; Proulx, D. Algae and waste water. *J. Appl. Phycol.* **1992**, *4*, 247–254. [CrossRef]
45. European Commission on Environmnet. *Heavy Metals in Wastes*; COWI A/S: Copenhagen, Denmark, 2002.
46. Chinnasamy, S.; Bhatnagar, A.; Hunt, R.W.; Das, K.C. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresour. Technol.* **2010**, *101*, 3097–3105. [CrossRef] [PubMed]
47. Henze, M.; Harremoes, P.; la Cour Jansen, J.; Arvin, E. *Wastewater Treatment: Biological and Chemical Processes*; Springer Science & Business Media: Berlin, Germany, 2001.
48. Westholm, L.J. Substrates for phosphorus removal—Potential benefits for on-site wastewater treatment? *Water Res.* **2006**, *40*, 23–36. [CrossRef] [PubMed]
49. De-Bashan, L.E.; Bashan, Y. Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003). *Water Res.* **2004**, *38*, 4222–4246. [CrossRef] [PubMed]
50. Sutton, M.A.; Oenema, O.; Erisman, J.W.; Leip, A.; van Grinsven, H.; Winiwarter, W. Too much of a good thing. *Nature* **2011**, *472*, 159–161. [CrossRef] [PubMed]
51. Cordell, D.; Drangert, J.-O.; White, S. The story of phosphorus: Global food security and food for thought. *Glob. Environ. Chang.* **2009**, *19*, 292–305. [CrossRef]
52. Clarens, A.F.; Resurreccion, E.P.; White, M.A.; Colosi, L.M. Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environ. Sci. Technol.* **2010**, *44*, 1813–1819. [CrossRef] [PubMed]
53. Wang, L.; Min, M.; Li, Y.; Chen, P.; Chen, Y.; Liu, Y.; Wang, Y.; Ruan, R. Cultivation of green algae *Chlorella* sp. In different wastewaters from municipal wastewater treatment plant. *Appl. Biochem. Biotechnol.* **2010**, *162*, 1174–1186. [CrossRef] [PubMed]
54. McGinn, P.J.; Dickinson, K.E.; Bhatti, S.; Frigon, J.C.; Guiot, S.R.; O'Leary, S.J. Integration of microalgae cultivation with industrial waste remediation for biofuel and bioenergy production: Opportunities and limitations. *Photosynth. Res.* **2011**, *109*, 231–247. [CrossRef] [PubMed]
55. Christenson, L.; Sims, R. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnol. Adv.* **2011**, *29*, 686–702. [CrossRef] [PubMed]
56. Larsdotter, K. Wastewater treatment with microalgae-a literature review. *Vatten* **2006**, *62*, 31.
57. Park, J.; Craggs, R.; Shilton, A. Wastewater treatment high rate algal ponds for biofuel production. *Bioresour. Technol.* **2011**, *102*, 35–42. [CrossRef] [PubMed]
58. Cai, T.; Park, S.Y.; Li, Y. Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renew. Sust. Energy Rev.* **2013**, *19*, 360–369. [CrossRef]
59. Stumm, W.; Morgan, J.J. *Aquatic Chemistry; an Introduction Emphasizing Chemical Equilibria in Natural Waters*; Willey-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 1970.
60. Chisti, Y. Constraints to commercialization of algal fuels. *J. Biotechnol.* **2013**, *167*, 201–214. [CrossRef] [PubMed]
61. Powell, N.; Shilton, A.; Chisti, Y.; Pratt, S. Towards a luxury uptake process via microalgae—Defining the polyphosphate dynamics. *Water Res.* **2009**, *43*, 4207–4213. [CrossRef] [PubMed]
62. Benvenuti, G.; Bosma, R.; Cuaresma, M.; Janssen, M.; Barbosa, M.J.; Wijffels, R.H. Selecting microalgae with high lipid productivity and photosynthetic activity under nitrogen starvation. *J. Appl. Phycol.* **2015**, *27*, 1425–1431. [CrossRef]
63. Breuer, G.; Martens, D.E.; Draaisma, R.B.; Wijffels, R.H.; Lamers, P.P. Photosynthetic efficiency and carbon partitioning in nitrogen-starved *Scenedesmus obliquus*. *Algal Res.* **2015**, *9*, 254–262. [CrossRef]
64. Cheah, W.Y.; Show, P.L.; Chang, J.-S.; Ling, T.C.; Juan, J.C. Biosequestration of atmospheric CO_2 and flue gas-containing CO_2 by microalgae. *Bioresour. Technol.* **2015**, *184*, 190–201. [CrossRef] [PubMed]
65. Van Den Hende, S.; Vervaeren, H.; Boon, N. Flue gas compounds and microalgae: (Bio-)chemical interactions leading to biotechnological opportunities. *Biotechnol. Adv.* **2012**, *30*, 1405–1424. [CrossRef] [PubMed]

66. Steeneveldt, R.; Berger, B.; Torp, T. CO_2 capture and storage: Closing the knowing–doing gap. *Chem. Eng. Res. Des.* **2006**, *84*, 739–763. [CrossRef]
67. Kanniche, M.; Bouallou, C. CO_2 capture study in advanced integrated gasification combined cycle. *Appl. Therm. Eng.* **2007**, *27*, 2693–2702. [CrossRef]
68. Svensson, R.; Odenberger, M.; Johnsson, F.; Strömberg, L. Transportation systems for CO_2—-Application to carbon capture and storage. *Energy Convers. Manag.* **2004**, *45*, 2343–2353. [CrossRef]
69. Berberoglu, H.; Gomez, P.S.; Pilon, L. Radiation characteristics of *Botryococcus braunii*, *Chlorococcum littorale*, and *Chlorella* sp. Used for CO_2 fixation and biofuel production. *J. Quant. Spectrosc. Radiat. Transf.* **2009**, *110*, 1879–1893. [CrossRef]
70. Yamasaki, A. An overview of CO_2 mitigation options for global warming—Emphasizing CO_2 sequestration options. *J. Chem. Eng. Japan* **2003**, *36*, 361–375. [CrossRef]
71. Richmond, A. *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*; John Wiley & Sons: Oxford, UK, 2008.
72. Chojnacka, K.; Marquez-Rocha, F.-J. Kinetic and stoichiometric relationships of the energy and carbon metabolism in the culture of microalgae. *Biotechnology* **2004**, *3*, 21–34.
73. Markou, G.; Angelidaki, I.; Georgakakis, D. Microalgal carbohydrates: An overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels. *Appl. Microbiol. Biotechnol.* **2012**, *96*, 631–645. [CrossRef] [PubMed]
74. Cheirsilp, B.; Torpee, S. Enhanced growth and lipid production of microalgae under mixotrophic culture condition: Effect of light intensity, glucose concentration and fed-batch cultivation. *Bioresour. Technol.* **2012**, *110*, 510–516. [CrossRef] [PubMed]
75. Zhan, J.; Rong, J.F.; Wang, Q. Mixotrophic cultivation, a preferable microalgae cultivation mode for biomass/bioenergy production, and bioremediation, advances and prospect. *Int. J. Hydrog. Energy* **2017**, *42*, 8505–8517. [CrossRef]
76. Izumo, A.; Fujiwara, S.; Oyama, Y.; Satoh, A.; Fujita, N.; Nakamura, Y.; Tsuzuki, M. Physicochemical properties of starch in chlorella change depending on the CO_2 concentration during growth: Comparison of structure and properties of pyrenoid and stroma starch. *Plant Sci.* **2007**, *172*, 1138–1147. [CrossRef]
77. Brányiková, I.; Maršálková, B.; Doucha, J.; Brányik, T.; Bišová, K.; Zachleder, V.; Vítová, M. Microalgae—Novel highly efficient starch producers. *Biotechnol. Bioeng.* **2011**, *108*, 766–776. [CrossRef] [PubMed]
78. El-Sheekh, M.; Abomohra, A.E.-F.; El-Azim, M.A.; Abou-Shanab, R. Effect of temperature on growth and fatty acids profile of the biodiesel producing microalga *Scenedesmus acutus*. *Biotechnol. Agron. Soc. Environ.* **2017**, *21*, 233–239.
79. Kessler, E. Comparative physiology, biochemistry, and the taxonomy of Chlorella (chlorophyceae). *Plant Syst. Evol.* **1976**, *125*, 129–138. [CrossRef]
80. Beherepatil, K.; Deore, L. Genus *Scenedesmus* from different habitats of nashik and it's environs (ms) india. *Int. J. Bioassays* **2013**, *2*, 727–734.
81. De Godos, I.; Arbib, Z.; Lara, E.; Rogalla, F. Evaluation of high rate algae ponds for treatment of anaerobically digested wastewater: Effect of CO_2 addition and modification of dilution rate. *Bioresour. Technol.* **2016**, *220*, 253–261. [CrossRef] [PubMed]
82. Novoveská, L.; Zapata, A.K.; Zabolotney, J.B.; Atwood, M.C.; Sundstrom, E.R. Optimizing microalgae cultivation and wastewater treatment in large-scale offshore photobioreactors. *Algal Res.* **2016**, *18*, 86–94. [CrossRef]
83. Shen, L.; Ndayambaje, J.D.; Murwanashyaka, T.; Cui, W.; Manirafasha, E.; Chen, C.; Wang, Y.; Lu, Y. Assessment upon heterotrophic microalgae screened from wastewater microbiota for concurrent pollutants removal and biofuel production. *Bioresour. Technol.* **2017**, *245*, 386–393. [CrossRef] [PubMed]
84. Razzak, S.A.; Hossain, M.M.; Lucky, R.A.; Bassi, A.S.; de Lasa, H. Integrated CO_2 capture, wastewater treatment and biofuel production by microalgae culturing—A review. *Renew. Sust. Energy Rev.* **2013**, *27*, 622–653. [CrossRef]
85. Vasudevan, P.T.; Briggs, M. Biodiesel production—Current state of the art and challenges. *J. Ind. Microbiol. Biotechnol.* **2008**, *35*, 421. [CrossRef] [PubMed]

86. Jimenez-Perez, M.; Sanchez-Castillo, P.; Romera, O.; Fernandez-Moreno, D.; Pérez-Martınez, C. Growth and nutrient removal in free and immobilized planktonic green algae isolated from pig manure. *Enzyme Microb. Technol.* **2004**, *34*, 392–398. [CrossRef]
87. Zhou, W.; Chen, P.; Min, M.; Ma, X.; Wang, J.; Griffith, R.; Hussain, F.; Peng, P.; Xie, Q.; Li, Y. Environment-enhancing algal biofuel production using wastewaters. *Renew. Sust. Energy Rev.* **2014**, *36*, 256–269. [CrossRef]
88. Moreno-Garrido, I.; Canavate, J. Assessing chemical compounds for controlling predator ciliates in outdoor mass cultures of the green algae *Dunaliella salina*. *Aquacult. Eng.* **2001**, *24*, 107–114. [CrossRef]
89. Richardson, J.W.; Johnson, M.D.; Zhang, X.; Zemke, P.; Chen, W.; Hu, Q. A financial assessment of two alternative cultivation systems and their contributions to algae biofuel economic viability. *Algal Res.* **2014**, *4*, 96–104. [CrossRef]
90. Chen, G.; Zhao, L.; Qi, Y. Enhancing the productivity of microalgae cultivated in wastewater toward biofuel production: A critical review. *Appl. Energy* **2015**, *137*, 282–291. [CrossRef]
91. Ren, H.; Tuo, J.; Addy, M.M.; Zhang, R.; Lu, Q.; Anderson, E.; Chen, P.; Ruan, R. Cultivation of *Chlorella vulgaris* in a pilot-scale photobioreactor using real centrate wastewater with waste glycerol for improving microalgae biomass production and wastewater nutrients removal. *Bioresour. Technol.* **2017**, *245*, 1130–1138. [CrossRef] [PubMed]
92. Fernández-Linares, L.C.; Barajas, C.G.; Páramo, E.D.; Corona, J.A.B. Assessment of *Chlorella vulgaris* and indigenous microalgae biomass with treated wastewater as growth culture medium. *Bioresour. Technol.* **2017**, *244*, 400–406. [CrossRef] [PubMed]
93. Benvenuti, G.; Bosma, R.; Klok, A.J.; Ji, F.; Lamers, P.P.; Barbosa, M.J.; Wijffels, R.H. Microalgal triacylglycerides production in outdoor batch-operated tubular pbrs. *Biotechnol. Biofuels* **2015**, *8*, 100. [CrossRef] [PubMed]
94. Wu, J.-Y.; Lay, C.-H.; Chen, C.-C.; Wu, S.-Y. Lipid accumulating microalgae cultivation in textile wastewater: Environmental parameters optimization. *J. Taiwan Inst. Chem. Eng.* **2017**, *79*, 1–6. [CrossRef]
95. Álvarez-Díaz, P.; Ruiz, J.; Arbib, Z.; Barragán, J.; Garrido-Pérez, M.; Perales, J. Freshwater microalgae selection for simultaneous wastewater nutrient removal and lipid production. *Algal Res.* **2017**, *24*, 477–485. [CrossRef]
96. Rinna, F.; Buono, S.; Cabanelas, I.T.D.; Nascimento, I.A.; Sansone, G.; Barone, C.M.A. Wastewater treatment by microalgae can generate high quality biodiesel feedstock. *J. Water Process. Eng.* **2017**, *18*, 144–149. [CrossRef]
97. Lau, P.; Tam, N.; Wong, Y. Effect of algal density on nutrient removal from primary settled wastewater. *Environ. Pollut.* **1995**, *89*, 59–66. [CrossRef]
98. Martınez, M.; Sánchez, S.; Jimenez, J.; El Yousfi, F.; Munoz, L. Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. *Bioresour. Technol.* **2000**, *73*, 263–272. [CrossRef]
99. Ruiz-Marin, A.; Mendoza-Espinosa, L.G.; Stephenson, T. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresour. Technol.* **2010**, *101*, 58–64. [CrossRef] [PubMed]
100. Di Termini, I.; Prassone, A.; Cattaneo, C.; Rovatti, M. On the nitrogen and phosphorus removal in algal photobioreactors. *Ecol. Eng.* **2011**, *37*, 976–980. [CrossRef]
101. Gentili, F.G. Microalgal biomass and lipid production in mixed municipal, dairy, pulp and paper wastewater together with added flue gases. *Bioresour. Technol.* **2014**, *169*, 27–32. [CrossRef] [PubMed]
102. Olguín, E.J.; Galicia, S.; Mercado, G.; Pérez, T. Annual productivity of spirulina (arthrospira) and nutrient removal in a pig wastewater recycling process under tropical conditions. *J. Appl. Phycol.* **2003**, *15*, 249–257. [CrossRef]
103. An, J.-Y.; Sim, S.-J.; Lee, J.S.; Kim, B.W. Hydrocarbon production from secondarily treated piggery wastewater by the green alga *Botryococcus braunii*. *J. Appl. Phycol.* **2003**, *15*, 185–191. [CrossRef]
104. Dumas, A.; Laliberte, G.; Lessard, P.; De La Noüe, J. Biotreatment of fish farm effluents using the cyanobacterium *Phormidium bohneri*. *Aquacult. Eng.* **1998**, *17*, 57–68. [CrossRef]
105. Yewalkar-Kulkarni, S.; Gera, G.; Nene, S.; Pandare, K.; Kulkarni, B.; Kamble, S. Exploiting phosphate-starved cells of *Scenedesmus* sp. For the treatment of raw sewage. *Indian J. Microbiol.* **2017**, *57*, 241–249. [CrossRef] [PubMed]

106. Úbeda, B.; Gálvez, J.Á.; Michel, M.; Bartual, A. Microalgae cultivation in urban wastewater: *Coelastrum cf. pseudomicroporum* as a novel carotenoid source and a potential microalgae harvesting tool. *Bioresour. Technol.* **2017**, *228*, 210–217. [CrossRef] [PubMed]
107. De Godos, I.; Blanco, S.; García-Encina, P.A.; Becares, E.; Muñoz, R. Influence of flue gas sparging on the performance of high rate algae ponds treating agro-industrial wastewaters. *J. Hazard. Mater.* **2010**, *179*, 1049–1054. [CrossRef] [PubMed]
108. Shen, Y.; Yuan, W.; Pei, Z.; Mao, E. Culture of microalga *Botryococcus* in livestock wastewater. *Trans. ASABE* **2008**, *51*, 1395–1400. [CrossRef]
109. Kothari, R.; Pathak, V.V.; Kumar, V.; Singh, D. Experimental study for growth potential of unicellular alga *Chlorella pyrenoidosa* on dairy waste water: An integrated approach for treatment and biofuel production. *Bioresour. Technol.* **2012**, *116*, 466–470. [CrossRef] [PubMed]
110. Gonzalez, C.; Marciniak, J.; Villaverde, S.; Leon, C.; Garcia, P.A.; Munoz, R. Efficient nutrient removal from swine manure in a tubular biofilm photo-bioreactor using algae-bacteria consortia. *Water Sci. Technol.* **2008**, *58*, 95–102. [CrossRef] [PubMed]
111. Qi, W.; Chen, T.; Wang, L.; Wu, M.; Zhao, Q.; Wei, W. High-strength fermentable wastewater reclamation through a sequential process of anaerobic fermentation followed by microalgae cultivation. *Bioresour. Technol.* **2017**, *227*, 317–323. [CrossRef] [PubMed]
112. Liu, H.; Lu, Q.; Wang, Q.; Liu, W.; Wei, Q.; Ren, H.; Ming, C.; Min, M.; Chen, P.; Ruan, R. Isolation of a bacterial strain, *Acinetobacter* sp. From centrate wastewater and study of its cooperation with algae in nutrients removal. *Bioresour. Technol.* **2017**, *235*, 59–69. [CrossRef] [PubMed]
113. Lin, C.-Y.; Nguyen, M.-L.T.; Lay, C.-H. Starch-containing textile wastewater treatment for biogas and microalgae biomass production. *J. Clean. Prod.* **2017**, *168*, 331–337. [CrossRef]
114. Da Fontoura, J.T.; Rolim, G.S.; Farenzena, M.; Gutterres, M. Influence of light intensity and tannery wastewater concentration on biomass production and nutrient removal by microalgae *Scenedesmus* sp. *Process. Saf. Environ.* **2017**, *111*, 355–362. [CrossRef]
115. Abid, A.; Saidane, F.; Hamdi, M. Feasibility of carbon dioxide sequestration by *Spongiochloris* sp. microalgae during petroleum wastewater treatment in airlift bioreactor. *Bioresour. Technol.* **2017**, *234*, 297–302. [CrossRef] [PubMed]
116. Reyimu, Z.; Özçimen, D. Batch cultivation of marine microalgae *Nannochloropsis oculata* and *Tetraselmis suecica* in treated municipal wastewater toward bioethanol production. *J. Clean. Prod.* **2017**, *150*, 40–46. [CrossRef]
117. Brennan, L.; Owende, P. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sust. Energy Rev.* **2010**, *14*, 557–577. [CrossRef]
118. Sutherland, D.L. Enhancing the Performance of Wastewater Microalgae through Chemical and Physical Modifications in High Rate Algal Ponds. Ph.D. Thesis, University of Canterbury, Christchurch, New Zealand, 2015.
119. Becker, E.W. *Microalgae: Biotechnology and Microbiology*; Cambridge University Press: Cambridge, UK, 1994; Volume 10.
120. Pulz, O. Photobioreactors: Production systems for phototrophic microorganisms. *Appl. Microbiol. Biotechnol.* **2001**, *57*, 287–293. [PubMed]
121. Acién Fernández, F.G.; González-López, C.V.; Fernández Sevilla, J.M.; Molina Grima, E. Conversion of CO_2 into biomass by microalgae: How realistic a contribution may it be to significant CO_2 removal? *Appl. Microbiol. Biotechnol.* **2012**, *96*, 577–586. [CrossRef] [PubMed]
122. Trozzi, C.; Rentz, O.; Oertel, D.; Woodfield, M.; Stewart, R. *Energy Industries. Combustion in Energy and Transformation Industries. Air Pollutant Emission Inventory Guidebook*; European Environment Agency: Copenhagen, Denmark, 2010.
123. Niessen, W.R. *Combustion and Incineration Processes: Applications in Environmental Engineering*; CRC Press Taylor & Francis Group: Boca Raton, FL, USA, 2010.
124. Ji, M.-K.; Yun, H.-S.; Park, Y.-T.; Kabra, A.N.; Oh, I.-H.; Choi, J. Mixotrophic cultivation of a microalga *Scenedesmus obliquus* in municipal wastewater supplemented with food wastewater and flue gas CO_2 for biomass production. *J. Environ. Manag.* **2015**, *159*, 115–120. [CrossRef] [PubMed]
125. Riebesell, U.; Wolf-Gladrow, D.A.; Smetacek, V. Carbon dioxide limitation of marine phytoplankton growth rates. *Nature* **1993**, *361*, 249. [CrossRef]

126. He, L.; Subramanian, V.R.; Tang, Y.J. Experimental analysis and model-based optimization of microalgae growth in photo-bioreactors using flue gas. *Biomass Bioenergy* **2012**, *41*, 131–138. [CrossRef]
127. Jiang, Y.; Zhang, W.; Wang, J.; Chen, Y.; Shen, S.; Liu, T. Utilization of simulated flue gas for cultivation of *Scenedesmus dimorphus*. *Bioresour. Technol.* **2013**, *128*, 359–364. [CrossRef] [PubMed]
128. Olaizola, M. Microalgal removal of CO_2 from flue gases: Changes in medium ph and flue gas composition do not appear to affect the photochemical yield of microalgal cultures. *Biotechnol. Bioprocess. Eng.* **2003**, *8*, 360–367. [CrossRef]
129. Chiu, S.-Y.; Kao, C.-Y.; Chen, C.-H.; Kuan, T.-C.; Ong, S.-C.; Lin, C.-S. Reduction of CO_2 by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. *Bioresour. Technol.* **2008**, *99*, 3389–3396. [CrossRef] [PubMed]
130. Tang, D.; Han, W.; Li, P.; Miao, X.; Zhong, J. CO_2 biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO_2 levels. *Bioresour. Technol.* **2011**, *102*, 3071–3076. [CrossRef] [PubMed]
131. Douskova, I.; Doucha, J.; Livansky, K.; Machat, J.; Novak, P.; Umysova, D.; Zachleder, V.; Vitova, M. Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. *Appl. Microbiol. Biotechnol.* **2009**, *82*, 179–185. [CrossRef] [PubMed]
132. La Rocca, N.; Andreoli, C.; Giacometti, G.M.; Rascio, N.; Moro, I. Responses of the antarctic microalga *Koliella antarctica* (trebouxiophyceae, chlorophyta) to cadmium contamination. *Photosynthetica* **2009**, *47*, 471–479. [CrossRef]
133. Hess, D.; Napan, K.; McNeil, B.T.; Torres, E.M.; Guy, T.; McLean, J.E.; Quinn, J.C. Quantification of effects of flue gas derived inorganic contaminants on microalgae growth system and end fate of contaminants. *Algal Res.* **2017**, *25*, 68–75. [CrossRef]
134. Greenwell, H.C.; Laurens, L.M.L.; Shields, R.J.; Lovitt, R.W.; Flynn, K.J. Placing microalgae on the biofuels priority list: A review of the technological challenges. *J. R. Soc. Interface* **2010**, *7*, 703–726. [CrossRef] [PubMed]
135. Yoo, C.; Jun, S.-Y.; Lee, J.-Y.; Ahn, C.-Y.; Oh, H.-M. Selection of microalgae for lipid production under high levels carbon dioxide. *Bioresour. Technol.* **2010**, *101*, S71–S74. [CrossRef] [PubMed]
136. Morais, M.G.; Costa, J.A.V. Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. *Energy Convers. Manag.* **2007**, *48*, 2169–2173. [CrossRef]
137. Yun, Y.S.; Lee, S.B.; Park, J.M.; Lee, C.I.; Yang, J.W. Carbon dioxide fixation by algal cultivation using wastewater nutrients. *J. Chem. Technol. Biotechnol.* **1997**, *69*, 451–455. [CrossRef]
138. Nayak, M.; Karemore, A.; Sen, R. Sustainable valorization of flue gas co2 and wastewater for the production of microalgal biomass as a biofuel feedstock in closed and open reactor systems. *RSC Adv.* **2016**, *6*, 91111–91120. [CrossRef]
139. Rivera-Utrilla, J.; Sánchez-Polo, M.; Ferro-García, M.Á.; Prados-Joya, G.; Ocampo-Pérez, R. Pharmaceuticals as emerging contaminants and their removal from water. A review. *Chemosphere* **2013**, *93*, 1268–1287. [CrossRef] [PubMed]
140. Solé, A.; Matamoros, V. Removal of endocrine disrupting compounds from wastewater by microalgae co-immobilized in alginate beads. *Chemosphere* **2016**, *164*, 516–523. [CrossRef] [PubMed]
141. Guo, W.-Q.; Zheng, H.-S.; Li, S.; Du, J.-S.; Feng, X.-C.; Yin, R.-L.; Wu, Q.-L.; Ren, N.-Q.; Chang, J.-S. Removal of cephalosporin antibiotics 7-aca from wastewater during the cultivation of lipid-accumulating microalgae. *Bioresour. Technol.* **2016**, *221*, 284–290. [CrossRef] [PubMed]
142. Geiger, E.; Hornek-Gausterer, R.; Saçan, M.T. Single and mixture toxicity of pharmaceuticals and chlorophenols to freshwater algae *Chlorella vulgaris*. *Ecotox Environ. Saf.* **2016**, *129*, 189–198. [CrossRef] [PubMed]
143. Bai, X.; Acharya, K. Removal of trimethoprim, sulfamethoxazole, and triclosan by the green alga *Nannochloris* sp. *J. Hazard. Mater.* **2016**, *315*, 70–75. [CrossRef] [PubMed]
144. Peng, F.-Q.; Ying, G.-G.; Yang, B.; Liu, S.; Lai, H.-J.; Liu, Y.-S.; Chen, Z.-F.; Zhou, G.-J. Biotransformation of progesterone and norgestrel by two freshwater microalgae (*Scenedesmus obliquus* and *Chlorella pyrenoidosa*): Transformation kinetics and products identification. *Chemosphere* **2014**, *95*, 581–588. [CrossRef] [PubMed]
145. Wang, S.; Wang, X.; Poon, K.; Wang, Y.; Li, S.; Liu, H.; Lin, S.; Cai, Z. Removal and reductive dechlorination of triclosan by *Chlorella pyrenoidosa*. *Chemosphere* **2013**, *92*, 1498–1505. [CrossRef] [PubMed]

146. Escapa, C.; Coimbra, R.; Paniagua, S.; García, A.; Otero, M. Nutrients and pharmaceuticals removal from wastewater by culture and harvesting of *Chlorella sorokiniana*. *Bioresour. Technol.* **2015**, *185*, 276–284. [CrossRef] [PubMed]
147. Gentili, F.G.; Fick, J. Algal cultivation in urban wastewater: An efficient way to reduce pharmaceutical pollutants. *J. Appl. Phycol.* **2017**, *29*, 255–262. [CrossRef] [PubMed]
148. Lima, S.A.; Raposo, M.F.J.; Castro, P.M.; Morais, R.M. Biodegradation of p-chlorophenol by a microalgae consortium. *Water Res.* **2004**, *38*, 97–102. [CrossRef] [PubMed]
149. Papazi, A.; Kotzabasis, K. "Rational" management of dichlorophenols biodegradation by the microalga *Scenedesmus obliquus*. *PLoS ONE* **2013**, *8*, e61682. [CrossRef]
150. Wilde, E.W.; Benemann, J.R. Bioremoval of heavy-metals by the use of microalgae. *Biotechnol. Adv.* **1993**, *11*, 781–812. [CrossRef]
151. Srivastava, N.; Majumder, C. Novel biofiltration methods for the treatment of heavy metals from industrial wastewater. *J. Hazard. Mater.* **2008**, *151*, 1–8. [CrossRef] [PubMed]
152. Gong, R.; Ding, Y.; Liu, H.; Chen, Q.; Liu, Z. Lead biosorption and desorption by intact and pretreated *Spirulina maxima* biomass. *Chemosphere* **2005**, *58*, 125–130. [CrossRef] [PubMed]
153. Abdel-Raouf, N.; Al-Homaidan, A.; Ibraheem, I. Microalgae and wastewater treatment. *Saudi J. Biol. Sci.* **2012**, *19*, 257–275. [CrossRef] [PubMed]
154. Abbas, S.H.; Ismail, I.M.; Mostafa, T.M.; Sulaymon, A.H. Biosorption of heavy metals: A review. *J. Chem. Sci. Technol.* **2014**, *3*, 74–102.
155. Muñoz, R.; Guieysse, B. Algal–bacterial processes for the treatment of hazardous contaminants: A review. *Water Res.* **2006**, *40*, 2799–2815. [CrossRef] [PubMed]
156. Moye, H.A.; Miles, C.J.; Phlips, E.J.; Sargent, B.; Merritt, K.K. Kinetics and uptake mechanisms for monomethylmercury between freshwater algae and water. *Environ. Sci. Technol.* **2002**, *36*, 3550–3555. [CrossRef] [PubMed]
157. Vilchez, C.; Garbayo, I.; Lobato, M.V.; Vega, J. Microalgae-mediated chemicals production and wastes removal. *Enzyme Microb. Technol.* **1997**, *20*, 562–572. [CrossRef]
158. Geoffroy, L.; Gilbin, R.; Simon, O.; Floriani, M.; Adam, C.; Pradines, C.; Cournac, L.; Garnier-Laplace, J. Effect of selenate on growth and photosynthesis of *Chlamydomonas reinhardtii*. *Aquat. Toxicol* **2007**, *83*, 149–158. [CrossRef] [PubMed]
159. Wu, Y.; Wang, W.-X. Accumulation, subcellular distribution and toxicity of inorganic mercury and methylmercury in marine phytoplankton. *Environ. Pollut.* **2011**, *159*, 3097–3105. [CrossRef] [PubMed]
160. Rodgher, S.; Espíndola, E.L.G.; Simões, F.C.F.; Tonietto, A.E. Cadmium and chromium toxicity to *Pseudokirchneriella subcapitata* and *Microcystis aeruginosa*. *Braz. Arch. Biol. Technol.* **2012**, *55*, 161–169. [CrossRef]
161. Carfagna, S.; Lanza, N.; Salbitani, G.; Basile, A.; Sorbo, S.; Vona, V. Physiological and morphological responses of lead or cadmium exposed *Chlorella sorokiniana* 211-8k (chlorophyceae). *SpringerPlus* **2013**, *2*, 147. [CrossRef] [PubMed]
162. Gómez-Jacinto, V.; García-Barrera, T.; Gómez-Ariza, J.L.; Garbayo-Nores, I.; Vílchez-Lobato, C. Elucidation of the defence mechanism in microalgae *Chlorella sorokiniana* under mercury exposure. Identification of hg–phytochelatins. *Chem. Biol. Interact.* **2015**, *238*, 82–90. [CrossRef] [PubMed]
163. Chang, H.-X.; Fu, Q.; Huang, Y.; Xia, A.; Liao, Q.; Zhu, X.; Zheng, Y.-P.; Sun, C.-H. An annular photobioreactor with ion-exchange-membrane for non-touch microalgae cultivation with wastewater. *Bioresour. Technol.* **2016**, *219*, 668–676. [CrossRef] [PubMed]
164. Katiyar, R.; Gurjar, B.; Biswas, S.; Pruthi, V.; Kumar, N.; Kumar, P. Microalgae: An emerging source of energy based bio-products and a solution for environmental issues. *Renew. Sust. Energy Rev.* **2017**, *72*, 1083–1093. [CrossRef]
165. Da Silva, M.J.; Cardoso, A.L. Heterogeneous tin catalysts applied to the esterification and transesterification reactions. *J. Catal.* **2013**, *2013*, 510509. [CrossRef]
166. Veillette, M.; Giroir-Fendler, A.; Faucheux, N.; Heitz, M. Esterification of free fatty acids with methanol to biodiesel using heterogeneous catalysts: From model acid oil to microalgae lipids. *Chem. Eng. J.* **2017**, *308*, 101–109. [CrossRef]
167. Orr, V.C.; Plechkova, N.V.; Seddon, K.R.; Rehmann, L. Disruption and wet extraction of the microalgae *Chlorella vulgaris* using room-temperature ionic liquids. *ACS Sustain. Chem. Eng.* **2016**, *4*, 591–600. [CrossRef]

168. Graboski, M.S.; McCormick, R.L. Combustion of fat and vegetable oil derived fuels in diesel engines. *Prog. Energy Combust. Sci.* **1998**, *24*, 125–164. [CrossRef]
169. Szybist, J.P.; Kirby, S.R.; Boehman, A.L. No x emissions of alternative diesel fuels: A comparative analysis of biodiesel and ft diesel. *Energy Fuels* **2005**, *19*, 1484–1492. [CrossRef]
170. Ji, M.-K.; Abou-Shanab, R.A.; Kim, S.-H.; Salama, E.-S.; Lee, S.-H.; Kabra, A.N.; Lee, Y.-S.; Hong, S.; Jeon, B.-H. Cultivation of microalgae species in tertiary municipal wastewater supplemented with CO_2 for nutrient removal and biomass production. *Ecol. Eng.* **2013**, *58*, 142–148. [CrossRef]
171. Raheem, A.; Azlina, W.W.; Yap, Y.T.; Danquah, M.K.; Harun, R. Thermochemical conversion of microalgal biomass for biofuel production. *Renew. Sust. Energy Rev.* **2015**, *49*, 990–999. [CrossRef]
172. Selvaratnam, T.; Pegallapati, A.; Montelya, F.; Rodriguez, G.; Nirmalakhandan, N.; Lammers, P.J.; Van Voorhies, W. Feasibility of algal systems for sustainable wastewater treatment. *Renew. Energy* **2015**, *82*, 71–76. [CrossRef]
173. Asomaning, J.; Omidghane, M.; Chae, M.; Bressler, D.C. Thermal processing of algal biomass for biofuel production. *Curr. Opin. Green Sustain. Chem.* **2016**, *2*, 1–5. [CrossRef]
174. Hallenbeck, P.; Grogger, M.; Mraz, M.; Veverka, D. Solar biofuels production with microalgae. *Appl. Energy* **2016**, *179*, 136–145. [CrossRef]
175. Zhu, Y.; Piotrowska, P.; van Eyk, P.J.; Boström, D.; Kwong, C.W.; Wang, D.; Cole, A.J.; de Nys, R.; Gentili, F.G.; Ashman, P.J. Cogasification of australian brown coal with algae in a fluidized bed reactor. *Energy Fuels* **2015**, *29*, 1686–1700. [CrossRef]
176. Chen, C.; Ma, X.; Liu, K. Thermogravimetric analysis of microalgae combustion under different oxygen supply concentrations. *Appl. Energy* **2011**, *88*, 3189–3196. [CrossRef]
177. Vassilev, S.V.; Vassileva, C.G. Composition, properties and challenges of algae biomass for biofuel application: An overview. *Fuel* **2016**, *181*, 1–33. [CrossRef]
178. Mu, D.; Min, M.; Krohn, B.; Mullins, K.A.; Ruan, R.; Hill, J. Life cycle environmental impacts of wastewater-based algal biofuels. *Environ. Sci. Technol.* **2014**, *48*, 11696–11704. [CrossRef] [PubMed]
179. Mehrabadi, A.; Craggs, R.; Farid, M.M. Pyrolysis of wastewater treatment high rate algal pond (wwt hrap) biomass. *Algal Res.* **2017**, *24*, 509–519. [CrossRef]
180. Grierson, S.; Strezov, V.; Shah, P. Properties of oil and char derived from slow pyrolysis of tetraselmis chui. *Bioresour. Technol.* **2011**, *102*, 8232–8240. [CrossRef] [PubMed]
181. Azizi, K.; Keshavarz Moraveji, M.; Abedini Najafabadi, H. A review on bio-fuel production from microalgal biomass by using pyrolysis method. *Renew. Sust. Energy Rev.* **2018**, *82*, 3046–3059. [CrossRef]
182. Ferreira, A.; Ribeiro, B.; Marques, P.A.; Ferreira, A.F.; Dias, A.P.; Pinheiro, H.M.; Reis, A.; Gouveia, L. *Scenedesmus obliquus* mediated brewery wastewater remediation and CO_2 biofixation for green energy purposes. *J. Clean. Prod.* **2017**, *165*, 1316–1327. [CrossRef]
183. Grierson, S.; Strezov, V.; Ellem, G.; Mcgregor, R.; Herbertson, J. Thermal characterisation of microalgae under slow pyrolysis conditions. *J. Anal. Appl. Pyrolysis* **2009**, *85*, 118–123. [CrossRef]
184. Biller, P.; Ross, A.B. Hydrothermal processing of algal biomass for the production of biofuels and chemicals. *Biofuels* **2012**, *3*, 603–623. [CrossRef]
185. Gollakota, A.; Kishore, N.; Gu, S. A review on hydrothermal liquefaction of biomass. *Renew. Sust. Energy Rev.* 2017. [CrossRef]
186. Yoo, G.; Park, M.S.; Yang, J.-W.; Choi, M. Lipid content in microalgae determines the quality of biocrude and energy return on investment of hydrothermal liquefaction. *Appl. Energy* **2015**, *156*, 354–361. [CrossRef]
187. Chen, W.-T.; Qian, W.; Zhang, Y.; Mazur, Z.; Kuo, C.-T.; Scheppe, K.; Schideman, L.C.; Sharma, B.K. Effect of ash on hydrothermal liquefaction of high-ash content algal biomass. *Algal Res.* **2017**, *25*, 297–306. [CrossRef]
188. Biller, P.; Sharma, B.K.; Kunwar, B.; Ross, A.B. Hydroprocessing of bio-crude from continuous hydrothermal liquefaction of microalgae. *Fuel* **2015**, *159*, 197–205. [CrossRef]
189. Cheng, F.; Cui, Z.; Chen, L.; Jarvis, J.; Paz, N.; Schaub, T.; Nirmalakhandan, N.; Brewer, C.E. Hydrothermal liquefaction of high-and low-lipid algae: Bio-crude oil chemistry. *Appl. Energy* **2017**, *206*, 278–292. [CrossRef]
190. Jazrawi, C.; Biller, P.; He, Y.; Montoya, A.; Ross, A.B.; Maschmeyer, T.; Haynes, B.S. Two-stage hydrothermal liquefaction of a high-protein microalga. *Algal Res.* **2015**, *8*, 15–22. [CrossRef]
191. Cantrell, K.B.; Ducey, T.; Ro, K.S.; Hunt, P.G. Livestock waste-to-bioenergy generation opportunities. *Bioresour. Technol.* **2008**, *99*, 7941–7953. [CrossRef] [PubMed]

192. Show, P.L.; Tang, M.S.; Nagarajan, D.; Ling, T.C.; Ooi, C.-W.; Chang, J.-S. A holistic approach to managing microalgae for biofuel applications. *Int. J. Mol. Sci.* **2017**, *18*, 215. [CrossRef] [PubMed]
193. Abdelaziz, A.E.; Leite, G.B.; Hallenbeck, P.C. Addressing the challenges for sustainable production of algal biofuels: II. Harvesting and conversion to biofuels. *Environ. Technol.* **2013**, *34*, 1807–1836. [CrossRef] [PubMed]
194. Bahadar, A.; Khan, M.B. Progress in energy from microalgae: A review. *Renew. Sust. Energy Rev.* **2013**, *27*, 128–148. [CrossRef]
195. Patzelt, D.J.; Hindersin, S.; Elsayed, S.; Boukis, N.; Kerner, M.; Hanelt, D. Hydrothermal gasification of *Acutodesmus obliquus* for renewable energy production and nutrient recycling of microalgal mass cultures. *J. Appl. Phycol.* **2015**, *27*, 2239–2250. [CrossRef]
196. Kodali, D.R. *Trans Fats Replacement Solutions*; AOCS Press, Elsevier: Urbana, IL, USA, 2014.
197. Van Damme, S.; Bram, S.; Contino, F. Comparison of biodiesel production scenarios with coproduction of triacetin according to energy and ghg emissions. *Energy Procedia* **2014**, *61*, 1852–1859. [CrossRef]
198. Samson, R.; Leduy, A. Biogas production from anaerobic digestion of *Spirulina maxima* algal biomass. *Biotechnol. Bioeng.* **1982**, *24*, 1919–1924. [CrossRef] [PubMed]
199. Golueke, C.G.; Oswald, W.J.; Gotaas, H.B. Anaerobic digestion of algae. *Appl. Microbiol.* **1957**, *5*, 47. [PubMed]
200. Prajapati, S.K.; Kaushik, P.; Malik, A.; Vijay, V.K. Phycoremediation coupled production of algal biomass, harvesting and anaerobic digestion: Possibilities and challenges. *Biotechnol. Adv.* **2013**, *31*, 1408–1425. [CrossRef] [PubMed]
201. Angelidaki, I.; Ahring, B. Thermophilic anaerobic digestion of livestock waste: The effect of ammonia. *Appl. Microbiol. Biotechnol.* **1993**, *38*, 560–564. [CrossRef]
202. Chen, Y.; Cheng, J.J.; Creamer, K.S. Inhibition of anaerobic digestion process: A review. *Bioresour. Technol.* **2008**, *99*, 4044–4064. [CrossRef] [PubMed]
203. Sialve, B.; Bernet, N.; Bernard, O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnol. Adv.* **2009**, *27*, 409–416. [CrossRef] [PubMed]
204. Yenigün, O.; Demirel, B. Ammonia inhibition in anaerobic digestion: A review. *Process. Biochem.* **2013**, *48*, 901–911. [CrossRef]
205. Shanmugam, P.; Horan, N. Optimising the biogas production from leather fleshing waste by co-digestion with MSW. *Bioresour. Technol.* **2009**, *100*, 4117–4120. [CrossRef] [PubMed]
206. Yen, H.-W.; Brune, D.E. Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresour. Technol.* **2007**, *98*, 130–134. [CrossRef] [PubMed]
207. Schwede, S.; Kowalczyk, A.; Gerber, M.; Span, R. Anaerobic co-digestion of the marine microalga *Nannochloropsis salina* with energy crops. *Bioresour. Technol.* **2013**, *148*, 428–435. [CrossRef] [PubMed]
208. Olsson, J.; Feng, X.M.; Ascue, J.; Gentili, F.G.; Shabiimam, M.A.; Nehrenheim, E.; Thorin, E. Co-digestion of cultivated microalgae and sewage sludge from municipal waste water treatment. *Bioresour. Technol.* **2014**, *171*, 203–210. [CrossRef] [PubMed]
209. González-Fernández, C.; Molinuevo-Salces, B.; García-González, M.C. Evaluation of anaerobic codigestion of microalgal biomass and swine manure via response surface methodology. *Appl. Energy* **2011**, *88*, 3448–3453. [CrossRef]
210. Prajapati, S.K.; Malik, A.; Vijay, V.K. Comparative evaluation of biomass production and bioenergy generation potential of *Chlorella* spp. Through anaerobic digestion. *Appl. Energy* **2014**, *114*, 790–797. [CrossRef]
211. Klassen, V.; Blifernez-Klassen, O.; Wibberg, D.; Winkler, A.; Kalinowski, J.; Posten, C.; Kruse, O. Highly efficient methane generation from untreated microalgae biomass. *Biotechnol. Biofuels* **2017**, *10*, 186. [CrossRef] [PubMed]
212. Mussgnug, J.H.; Klassen, V.; Schlüter, A.; Kruse, O. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. *J. Biotechnol.* **2010**, *150*, 51–56. [CrossRef] [PubMed]
213. Passos, F.; Solé, M.; García, J.; Ferrer, I. Biogas production from microalgae grown in wastewater: Effect of microwave pretreatment. *Appl. Energy* **2013**, *108*, 168–175. [CrossRef]
214. Passos, F.; Uggetti, E.; Carrère, H.; Ferrer, I. Pretreatment of microalgae to improve biogas production: A review. *Bioresour. Technol.* **2014**, *172*, 403–412. [CrossRef] [PubMed]
215. Hamouda, R.A.; Sherif, S.A.; Ghareeb, M.M. Bioethanol production by various hydrolysis and fermentation processes with micro and macro green algae. *Waste Biomass Valoriz.* **2017**. [CrossRef]

216. Boonprab, K.; Matsui, K.; Kataoka, N. Preliminary study on bioethanol from fresh water algae, *Cladophora glomerata* (sarai kai) by the fungus, *Monascus* sp. Np1. *J. Appl. Phycol.* **2017**. [CrossRef]
217. Shokrkar, H.; Ebrahimi, S.; Zamani, M. Bioethanol production from acidic and enzymatic hydrolysates of mixed microalgae culture. *Fuel* **2017**, *200*, 380–386. [CrossRef]
218. Ho, S.-H.; Chen, C.-Y.; Chang, J.-S. Effect of light intensity and nitrogen starvation on CO_2 fixation and lipid/carbohydrate production of an indigenous microalga scenedesmus obliquus cnw-n. *Bioresour. Technol.* **2012**, *113*, 244–252. [CrossRef] [PubMed]
219. Razzak, S.A.; Ali, S.A.M.; Hossain, M.M. Biological CO_2 fixation with production of microalgae in wastewater—A review. *Renew. Sust. Energy Rev.* **2017**, *76*, 379–390. [CrossRef]
220. Jin, C.; Yao, M.; Liu, H.; Chia-fon, F.L.; Ji, J. Progress in the production and application of n-butanol as a biofuel. *Renew. Sust. Energy Rev.* **2011**, *15*, 4080–4106. [CrossRef]
221. Wang, Y.; Ho, S.-H.; Yen, H.-W.; Nagarajan, D.; Ren, N.-Q.; Li, S.; Hu, Z.; Lee, D.-J.; Kondo, A.; Chang, J.-S. Current advances on fermentative biobutanol production using third generation feedstock. *Biotechnol. Adv.* **2017**, *35*, 1049–1059. [CrossRef] [PubMed]
222. Wang, Y.; Ho, S.-H.; Cheng, C.-L.; Nagarajan, D.; Guo, W.-Q.; Lin, C.; Li, S.; Ren, N.; Chang, J.-S. Nutrients and COD removal of swine wastewater with an isolated microalgal strain *Neochloris aquatica* cl-m1 accumulating high carbohydrate content used for biobutanol production. *Bioresour. Technol.* **2017**, *242*, 7–14. [CrossRef] [PubMed]
223. Gao, K.; Orr, V.; Rehmann, L. Butanol fermentation from microalgae-derived carbohydrates after ionic liquid extraction. *Bioresour. Technol.* **2016**, *206*, 77–85. [CrossRef] [PubMed]
224. Batista, A.P.; Ambrosano, L.; Graça, S.; Sousa, C.; Marques, P.A.; Ribeiro, B.; Botrel, E.P.; Neto, P.C.; Gouveia, L. Combining urban wastewater treatment with biohydrogen production—An integrated microalgae-based approach. *Bioresour. Technol.* **2015**, *184*, 230–235. [CrossRef] [PubMed]
225. Batista, A.P.; Moura, P.; Marques, P.A.; Ortigueira, J.; Alves, L.; Gouveia, L. *Scenedesmus obliquus* as feedstock for biohydrogen production by *Enterobacter aerogenes* and *Clostridium butyricum*. *Fuel* **2014**, *117*, 537–543. [CrossRef]
226. Shobana, S.; Kumar, G.; Bakonyi, P.; Saratale, G.D.; Al-Muhtaseb, A.a.H.; Nemestóthy, N.; Bélafi-Bakó, K.; Xia, A.; Chang, J.-S. A review on the biomass pretreatment and inhibitor removal methods as key-steps towards efficient macroalgae-based biohydrogen production. *Bioresour. Technol.* **2017**, *244*, 1341–1348. [CrossRef] [PubMed]
227. Liu, C.-H.; Chang, C.-Y.; Liao, Q.; Zhu, X.; Liao, C.-F.; Chang, J.-S. Biohydrogen production by a novel integration of dark fermentation and mixotrophic microalgae cultivation. *Int. J. Hydrog. Energy* **2013**, *38*, 15807–15814. [CrossRef]
228. Phanduang, O.; Lunprom, S.; Salakkam, A.; Reungsang, A. Anaerobic solid-state fermentation of bio-hydrogen from microalgal *Chlorella* sp. Biomass. *Int. J. Hydrog. Energy* **2017**, *42*, 9650–9659. [CrossRef]