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Review article

An assessment of heterotrophy and mixotrophy in *Scenedesmus* and its utilization in wastewater treatment

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ABSTRACT

Wastewater contains various macro and micronutrients essential for growth. Microalgae-based wastewater treatment offers an alternative process that couples nutrient uptake and the value-added production of a biofuels feedstock or other algal-derived products. In this review, we summarize previous efforts to cultivate the microalgae *Scenedesmus* sp. in different wastewater sources, and report on the effectiveness of nutrient and organic substrate removal, CO_2 mitigation, and resulting biomass productivities. As biomass productivity is a function of growth, we also investigated the extent of functional genomics resources that are available to study the regulation of central carbon metabolism in *Scenedesmus*.

1. Introduction

Population growth as well as industrialization and urbanization increase the production of wastewater, leading to water toxicity and eutrophication when released into aquatic ecosystems. Using microalgae for the removal of nutrients and other contaminants from wastewater (particularly secondary and tertiary wastewater effluent) in phototrophic systems decreases the risks of eutrophication, and simultaneously fixes CO_2 through photosynthesis, effectively reducing greenhouse gas (GHG) emissions [1–9]. Given that monosaccharides and other organic compounds are prevalent in many wastewater sources [10–13], microalgal growth in these waste streams may be considered mixotrophic, since they can utilize inorganic CO_2 for photosynthesis and organic carbon compounds for other metabolic pathways. Previously, microalgae have been cultivated mixotrophically in these waste streams [5,14–20]. Post-utilization in wastewater treatment applications, cells can be harvested for their lipid content (formed as byproducts of this procedure [15,16,21–23]), and extracted and converted into fatty acid methyl esters (FAMEs) by transesterification for use as biofuel. Depending on the water source, the remaining high-protein algal biomass may be used as feed supplements for the livestock and aquaculture industries [24,70]. A second cultivation scheme for microalgae is a heterotrophic system (closed system without light), which may result in increased cell density, modulated cell composition, and increased biomass productivity compared to phototrophic cultivation [11,25,26]. Examples are the green algae *Auxenochlorella protothecoides* and *Chromochloris zofingiensis*, which can shift their metabolism to grow under heterotrophic conditions [27,28].

Although microalgal growth and mineral nutrient removal from wastewater have been previously examined [29–32], the nature and level of organic carbon content in wastewater, as well as the uptake and utilization of these substrates by microalgae is not well understood. One microalgal freshwater genus that has been used in both biofuel and wastewater treatment research is *Scenedesmus*. Compared to other oleaginous microalgae, *Scenedesmus* species currently represent some of the best candidates for the production of biofuels and biomaterials [33,34]. *Scenedesmus* sp. lipid content ranges from about 10% to 50% of dry biomass weight, depending on the species and growth conditions [23,33,86,93]. Moreover, *Scenedesmus* sp. have gained interest as a source for various secondary metabolites, particularly carotenoids (e.g. α-carotene, β-carotene, lutein, etc.), since many species are capable of enhanced pigment production while simultaneously treating different types of wastewater [93,132–134]. *Scenedesmus* sp. have also been shown to assimilate glucose by heterotrophic or mixotrophic growth [35–37]. Additionally, *Scenedesmus* can effectively grow in a wide temperature range, a property beneficial for outdoor raceway cultivation [38]. They can efficiently fix CO_2 [8], while showing high tolerance to industrial flue gas [37,39].

So far, strain development for wastewater treatment or to maximize productivity of *Scenedesmus* (and other microalgae as well) in biofuel

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Table 1
Elemental composition of different wastewaters reviewed here. Not available (N/A) means values were not reported in the research paper.

	Municipal secondary effluent [4]	Municipal wastewater [70]	Poultry wastewater (Flocculated) [71]	Food wastewater [37]	Brewery wastewater [73]	Dairy wastewater [5]	Urban wastewater centrate [7]
Total nitrogen (TN)	11–14 mg L^{-1}	52.6 mg L^{-1}	N/A	725 mg L^{-1}	150 mg L^{-1}	86 mg L^{-1}	N/A
N-$NH_4{}^+$	N/A	52.2 mg L^{-1}	259.3 mg L^{-1}	N/A	N/A	N/A	628 mg L^{-1}
N-$NO_3{}^-$	N/A	0.4 mg L^{-1}	Not Detected	N/A	N/A	31 mg L^{-1}	7.2 mg L^{-1}
Total phosphorus (TP)	1–1.5 mg L^{-1}	N/A	N/A	62.5 mg L^{-1}	220 mg L^{-1}	N/A	N/A
P-$PO_4{}^{3-}$	N/A	8.47 mg L^{-1}	23.4 mg L^{-1}	N/A	N/A	8.7 mg L^{-1}	64 mg L^{-1}
Total sulfur (TS)	N/A	N/A	N/A	N/A	2200 mg L^{-1}	13 mg L^{-1}	N/A
Total suspended solids (TSS)	N/A	540 mg L^{-1}	100 mg L^{-1}	N/A	N/A	3830 mg L^{-1}	0.12 mg L^{-1}
Total solids (TS)	N/A	N/A	700 mg L^{-1}	N/A	N/A	N/A	N/A
Total organic carbon (TOC)	18–22 mg L^{-1}	N/A	N/A	10,173 mg L^{-1}	N/A	170 mg L^{-1}	182 mg L^{-1}
Maltose	N/A	N/A	N/A	N/A	860 mg L^{-1}	N/A	N/A
Chemical oxygen demand (COD)	N/A	400 mg L^{-1}	97 mg O_2 L^{-1}	N/A	N/A	655 mg L^{-1}	N/A
CO_2 concentrations	0.03–15%	N/A	N/A	5–14%	N/A	N/A	10%
pH	7.5	6.9	N/A	6	N/A	6.5	7.8
Salinity	N/A	0.4 mg L^{-1}	N/A	3.7%	N/A	N/A	N/A
Malt extract	N/A	N/A	N/A	N/A	1000 mg L^{-1}	N/A	N/A
Yeast extract	N/A	N/A	N/A	N/A	500 mg L^{-1}	N/A	N/A
Ethanol	N/A	N/A	N/A	N/A	2 mL L^{-1}	N/A	N/A

cultivation systems have been limited due to the lack of genomic information, knowledge of genetic regulation, or effective genome manipulation tools. Given that core metabolic pathways and algal genetic systems are highly variable across and within algal classes, knowledge gained from well-studied chlorophytes (i.e. *Chlamydomonas* and *Chlorella*) is not always applicable. Herein, we report on the state of knowledge regarding the utilization of *Scenedesmus* sp. in the treatment of wastewater and the impact of this cultivation method on biomass and lipid production for fuel conversion. We also review the heterotrophic/mixotrophic cultivation techniques of *Scenedesmus* microalgae by manipulation of carbon/nitrogen sources in the growth medium, aimed at increasing biomass productivity. Finally, given the availability of reduced organic molecules in various wastewaters and their potential to improve microalgal biomass productivity, we additionally review what is known about carbon metabolism in this industrially-relevant lineage.

2. Cultivation and remediation schemes

Given that wastewater can be sourced from domestic, municipal, chemical, and/or agro-industrial processes, the characteristics of these effluents can vary compositionally and temporally [16,44,45]. Nonetheless, wastewater effluents rich in inorganic nutrients can support microalgae growth. These effluents have been shown to support cultivaton of microalgae in: 1) closed photobioreactors (PBRs) [46–48], 2) shallow, paddlewheel-mixed, open raceway ponds often called high-rate ponds (HRPs), and 3) revolving attached biofilm (RAB) systems [130,131]. Both HRPs and RABs have been utilized for final tertiary treatment prior to the discharge of effluents into aquatic ecosystems [16,45,49–57], while PBRs have been envisioned to produce high-value food supplements and pigments [46–48]. Each cultivation system has advantages and disadvantages. Suspended microalgae cultivation in PBRs or HRPs is energy-intensive due to the movement of large amounts of water. HRPs are the least expensive to operate but prone to contamination with undesired organisms, and are marked by low biomass productivity relative to PBRs and RAB systems. In addition to effective nutrients removal and wastewater treatment, RAB systems can enhance CO_2 mass transfer, resulting in significant increases in areal biomass production rates.

Many groups have been working to optimize nutrient removal and cultivation processes of the aforementioned systems. Nutrient use/removal efficiency has been enhanced in all systems by CO_2 addition [50,58,59]. For example, bubbling CO_2-rich flue gas into ponds or PBRs doubled algal biomass productivity to 16–20 g m^{-2} d^{-1} [15,58,60–64]. When cultivating in wastewater that is rich in dissolved organic matter, aerobic bacterial decomposition can increase carbon availability and boost algal biomass productivity [15,58]. With respect to harvesting/dewatering, algae can be collected from RAB systems more economically than from PBRs and HRPs [130,131]. To reduce dewatering costs of PBRs and HRPs, strain choice alone can simplify harvesting as *Scenedesmus* sp. and a few other colonial microalgae can settle by gravity. Furthermore, others have tested bioflocculation schemes to improve settling after the nutrients in media become limited [16,48,58,60,61,65–67]. In summary, some cost-benefit analyses have been completed to evaluate each cultivation method. For the cultivation and biomass production of *S. obliquus* in wastewater, multi-purpose phycoremediation processes can be economically viable [70], this may be achieved through generating revenue after wastewater treatment while simultaneously producing algal feedstocks for fuel and fertilizer applications. Additionally, algal biofilm cultivation offers promising alternative approaches for cost-effective production of high-density biomass [130,131].

3. Cultivation of *Scenedesmus* sp. in wastewater from different sources

The cultivation and remediation potential of *Scenedesmus*-dominated systems have been evaluated in several types of wastewater (Tables 1 & 2). Using municipal wastewater effluents for the cultivation of *S. obliquus*, removal of N and P reached almost 100% after a few days, while total organic carbon (TOC) removal reached between 60% and 75% [4,68,69,94–104]. Simultaneously, biomass productivity was at ~578 mg L^{-1} d^{-1}, and lipid productivity reached about 17 mg L^{-1} d^{-1} at 5% CO_2 aeration. Under these conditions, however, the productivity of *S. obliquus*-dominated cultures declined within 7 days, as bacterial contamination increased [4]. More recently, uptake of nutrients from municipal wastewater, and simultaneous accumulation of lipids, carbohydrates, and proteins were analyzed in *S. obliquus*. Results confirmed nutrient removal efficiency of 81% $NH_4{}^+$, 100% NO_3, 94% $PO_4{}^{3-}$, and 71% chemical oxygen demand (COD) after 16 days cultivation. Following algal biomass harvesting, protein, carbohydrate, and lipid contents were 28.5%, 27.5%, and 26.5% of dry biomass weight, respectively [70]. The growth and community dynamics of microalgal biofilms, pre-conditioned with wastewater and heavily inoculated with *S. obliquus*, was also monitored over an extended period of 26 days.

Table 2

Biomass production and composition of different *Scenedesmus* sp. reviewed here. Not available (N/A) means values were not reported in the research paper.

Microalgae	Media	Biomass productivity	Lipids	Proteins	Carbohydrates	CO_2 level	Cultivation duration	Reference
S. obliquus	Municipal wastewater	578 mg L^{-1} d^{-1}	17% dry weight	19% dry weight	64% dry weight	5%	6 days	[4]
S. obliquus	Municipal wastewater	85 mg L − 1 d − 1[a]	26.5% dry weight	28.5% dry weight	27.5% dry weight	Ambient	16 days	[70]
S. obliquus	Poultry wastewater	N/A	27%	N/A	23%	Ambient	13 days	[71]
S. obliquus	Food wastewater	73 mg L^{-1} d^{-1}	11 mg L^{-1} d^{-1}	N/A	16 mg L^{-1} d^{-1}	10%	6 days	[37]
S. obliquus	Brewery wastewater	100 mg L^{-1} d^{-1}	27% dry weight	N/A	N/A	Ambient	9 days	[73]
Consortium w/ *Scenedesmus* sp.	10–15% municipal wastewater	2.64 g m^{-2} d^{-1}	6.82% dry weight	54.5% dry weight	8.98% dry weight	6%	12 days	[74]
S. quadricauda	Dairy wastewater	72mg L − 1 d − 1[a]	N/A	N/A	N/A	Ambient	8 days	[5]
Scenedesmus sp.	30% effluent urban wastewater	23 g m^{-2} d^{-1}	2.3 g m^{-2} d^{-1}	N/A	N/A	On demand	Semi-continuous	[7]

[a] Calculated based on the linear growth rate reported.

Despite heavy seeding, *S. obliquus* population declined while other chlorophytes (e.g. *Chlorella*) and cyanobacteria (e.g. *Leptolyngbya*) presumably carried with the wastewater surpassed the seed inoculum [105]. In other studies, *S. obliquus* was also grown in agroindustry poultry effluent using flocculated wastewater demonstrating 97% removal of both N and P [71]. *S. obliquus* grown mixotrophically in food wastewater supplied with real flue gas (10% CO_2) (Table 1) showed highest biomass growth, measured as dry weight (DW), at 0.44 g DW L^{-1} and total nitrogen removal at 22 mg L^{-1} after 6 days cultivation. Lipid and carbohydrate productivities were at 11 and 16 mg L^{-1} d^{-1}, respectively [37]. Furthermore, Hodaifa et al. [72] used N-deficient rinse water from olive oil extraction industry to grow *S. obliquus* [72]. This resulted in a major increase in mono and polyunsaturated fatty acids within the total lipid content extracted from the biomass. Lastly, brewery wastewater was also used to grow *S. obliquus* for biomass and lipid production [73,106]. Brewery wastewater containing large amounts of nutrients (Table 1) can cause serious environmental risks, if released without treatment. Under these conditions, *S. obliquus* biomass reached 0.9 g DW L^{-1}, while lipid content reached 27% of dry biomass weight after 9 days cultivation [73]. In brief, *S. obliquus* has been demonstrated to efficiently remove nutrients from a wide variety of wastewater streams.

A consortium of 15 microalgal isolates including *Scenedesmus* sp. was used to treat carpet industry effluents containing 10%–15% municipal sewage, under ambient air or high CO_2 (6%) levels [74]. Within 3 days, the consortium was able to assimilate almost 100% N and over 97% P from the growth medium. Algal protein content was high (~55%), while lipids and carbohydrates were low (Table 2) [74]. Mixed microalgal cultures comprising *Scenedesmus* sp. and *Chlorella* sp. also showed effective pesticides removal from agricultural wastewater effluents with maximum rates reaching ~100% for endosulfan and malathion, and ~75% for lindane and alachlor [107]. When utilizing *S. quadricauda* to treat dairy wastewater, this strain could grow in the culture medium and maximum biomass concentration reached within 8 days was about 0.5 g DW L^{-1}. Nutrient uptake including N, P, and TOC reached 86, 90, and 65%, respectively. Lipid analysis revealed a major increase in saturated fatty acids in *S. quadricauda* [5]. Other works investigated the growth of *Scenedesmus* sp. in synthetic wastewater or in effluents from the anaerobic digestion of urban wastewater, sparged with flue gas for on-demand CO_2 supply [7,108–110]. Under specific growth conditions of 30% effluent, *Scenedesmus* sp. biomass productivity reached 23 g m^{-2} d^{-1}, while lipid productivity reached 2.3 g m^{-2} d^{-1} (Table 2) [7]. Growth and uptake of organic coumpounds using *Scenedesmus* sp. were also tested in paper and oil mill industry wastewaters [111], as well as textile wastewater [112]. These studies showed high-efficiency uptake of butyrate, propionate, and acetate reaching 98%, 97% and 95%, respectively, while *Scenedesmus* showed a growth rate of 0.53 g d^{-1} [112]. Lastly, mutant strain *Scenedesmus* sp. Z-4 [113], grown in molasses wastewater, showed lipid productivity of ~95 mg L^{-1} d^{-1}, and significant N and P removal reaching 90.5% and 88.6%, respectively [114].

The potential for biosorption and heavy metals uptake from tannery wastewater was tested using *Scenedesmus* sp., under laboratory conditions. These studies showed ~98% uptake of copper (Cu), zinc (Zn), chromium (Cr), and lead (Pb) from the growth media [115]. It was also shown that Pb accumulation in *Acutodesmus (Scenedesmus) obliquus* resulted in cell toxicity and reduction in pigment, protein, and monosaccharide contents [116]. Similarly, Cr(VI) removal by the microalga *S. incrassatulus* was also evaluated in PBRs using synthetic effluents. Results from 2 studies show ~53% [75], or ~45% [76] Cr(VI)-removal efficiency, and a slight increase in the alga dry biomass. Cr(VI) is a cancer-causing agent produced through industrial processes, including leather tanning [76–78], and may be released into aquatic systems with generated wastewaters. Interestingly, under some conditions, more efficient inorganic nutrient uptake can occur when *Scenedesmus* sp. are co-cultured with other algae [75,79,117–122], bacteria [123–125], or fungi [126], while others showed that single species used in the treatment of domestic wastewaters were more productive [7,20,70].

4. Heterotrophic and mixotrophic growth of *Scenedesmus* sp

As previously mentioned, microalgae can utilize organic substrates (monosaccharides, amino acids, or other exogenous compounds), and can shift between phototrophic, heterotrophic or mixotrophic growth [13,80]. However, the type and quantity of substrates that are utilized in mixed waste streams have not been investigated and most (if not all) of what is known about organic carbon utilization by *Scenedesmus* has been from laboratory studies with defined medium. The growth rate of phototrophic *S. obliquus* cells increased under heterotrophic growth conditions in the dark, in the presence of 1% glucose as the sole carbon source [81]. Moreover, *S. obliquus* cells grown mixotrophically in 1.5% glucose increased their lipid productivity to 270 mg L^{-1} d^{-1}, which was 50-fold higher than in phototrophically-grown cells [82]. When *S. obliquus* was grown mixotrophically in minimal media (BG-11) containing xylose (4 g L^{-1}), cell density was ~3-fold higher than under phototrophic conditions [83]. Growth and biomass productivity of *S. obliquus* were also assessed in minimal media supplied with various concentrations of sodium bicarbonate, sodium chloride/sea water, glycerol, or sugarcane molasses. Among these growth conditions, high salinity (0.94 g L^{-1} NaCl concentration or 25% sea water) resulted in the highest increase in growth and fatty acid content [36]. The observed increase in lipid content in *S. obliquus* at higher salinity levels was confirmed in other reports [84,85].

Supplementing phototrophic or mixotrophic cultures of *Scenedesmus* with high concentrations of CO_2 can increase photosynthetic capacity and carbon fixation, while reaching higher biomass productivity [87,127]. Cultures of *Scenedesmus* sp. supplied with flue gas containing 5.5% CO_2 reached a biomass productivity of 203 mg L^{-1} d^{-1} and lipid productivity of 39 mg L^{-1} d^{-1}, which corresponds to about 18% lipid content [88]. In other studies, the highest growth rate of the microalga *S. dimorphus* was achieved with glucose at pH 5 under both heterotrophic and mixotrophic conditions. Nevertheless, not all organic carbon sources promote biomass productivity equally as cell growth gradually decreased when minimal medium was supplied with fructose, acetic acid, or proteose peptone [89]. Finally, biomass productivity of *S. bijuga* was evaluated under phototrophic, mixotrophic, and heterotrophic growth conditions [90]. During mixotrophic growth with 1% glucose, *S. bijuga* biomass increased ~6-fold compared to phototrophic growth, and ~4.5-fold compared to heterotrophic growth [90]. In short, a scant number of substrates have been tested for optimal mixotrophic or heterotrophic growth of a select number of *Scenedesmus* species. Collectively, biomass productivity in these species were generally much higher under mixotrophic conditions. However, the interplay between the different carbon sources and molecular mechanisms of carbon partitioning under these conditions is still unknown.

Scenedesmus central carbon metabolism

Understanding the molecular mechanisms of inorganic/organic carbon assimilation and sequestration, as well as advancing our knowledge of metabolic and gene regulatory networks will provide useful hypotheses for future strain development, as well as improvement of wastewater treatment efficacy and cultivation conditions to maximize algal biomass productivity. However, metabolic pathways in different microalgae can be flexible and diverse. Currently, a major gap in knowledge exists regarding the regulation of carbon fixation/uptake and partitioning in *Scenedesmus* sp. and other green algae. Moreover, the genetic basis for *Scenedesmus* growth response, as well as the regulatory and metabolic pathways governing cultivation in organic-rich wastewaters are largely unknown. Starch appears to be the preferred storage molecule under control, non-stress conditions, but under stress conditions, and particularly nutrient stress, starch and lipid biosynthetic pathways compete for common precursors, such as Acetyl-CoA the main precursor of fatty acid synthesis, resulting in major accumulation of storage neutral lipids. Most of the other biochemical pathways remain uncharacterized. To gain this knowledge, a few researchers have sequenced the genomes and transcriptomes of a small number of *Scenedesmus* species chosen for their potential as biofuel production platforms. The genomes of *S. obliquus* DOE0152z, *S. quadricauda* LWG002611, and *Tetradesmus* (*Scenedesmus*) *obliquus* UTEX393 were sequenced to identify the metabolic and regulatory pathways relevant to biofuel production [33,40,41]. Using the annotated genome of *S. quadricauda*, metabolic pathways relevant to carbon fixation and partitioning were reconstructed [33], revealing the presence of gene homologs encoding for enzymes active in alternative CO_2 uptake, as well as enzymes active in lipid and triacylglycerol (TAG) metabolism. Furthermore, using the draft assembly of the nuclear sequence from *T. obliquus* [41], core metabolic pathways essential for biomass formation, and energy production and maintenance were recently reconstructed for this strain [91].

Transcriptome analyses of the gene expression were also performed in a few *Scenedesmus* species to investigate the regulation mechanisms under conditions that trigger carbon partitioning to lipid and starch accumulation. Carreres et al. [43] compared the transcriptional changes in wild-type *T. obliquus* and a starchless mutant (*slm1*) growing in photobioreactors, under diurnal (16 h:8 h light:dark cycle) growth conditions. They found significant changes in expression of genes encoding for enzymes involved in carbohydrate metabolism under the analyzed conditions. In addition, starch deficiency in *slm1* resulted in impairments in energy production under dark conditions [43]. Nitrogen deprivation in the non-model microalga *S. acutus* resulted in the enhancement of lipid accumulation [42]. Differential gene expression analyses revealed the downregulation of genes involved in photosynthesis, TAG degradation, including several TAG lipases, as well as genes involved in starch synthesis. On the other hand, genes involved in starch degradation and glycolysis were upregulated [42]. Transcriptome analysis in *Scenedesmus* sp. was also performed under phosphorus starvation condition. Low P induced lipid accumulation, while carbohydrate content decreased [34]. The differential gene expression was consistent with these observations since genes encoding for diacylglycerol acyltransferase (DGAT) and pyruvate kinase were induced, and genes encoding for starch synthase and 1,4-α-glucan branching enzyme were downregulated [34]. Finally, a comparative transcriptomic analysis between *S. dimorphus* and *S. quadricauda* revealed differential lipid accumulation among these two species [92]. Results showed upregulation of genes involved in photosynthesis and carbon fixation in *S. dimorphus* compared to *S. quadricauda*. This may have induced higher lipid biosynthesis and accumulation in *S. dimorphus*. *Phosphoenolpyruvate Carboxylase* (*PEPC*) expression in the transcriptome of *S. dimorphus* was shown to be downregulated, while enolase, which catalyzes the production of phosphoenolpyruvate was induced [92]. Genes encoding for enzymes involved in the synthesis of Acetyl-CoA, as well as TAG biosynthesis were highly induced in *S. dimorphus* [92].

In summary, progress has been made towards defining the core carbon metabolic network in *Scendesmus*. However, the metabolic pathways regulating the uptake of different organic compounds available from wastewater remain to be studied and validated experimentally. In addition, regulatory mechanisms governing carbon partitioning have not yet been identified, and the interplay between autotrophic and heterotrophic carbon sources has not been investigated. It is not known, for example, if the regulatory mechanisms involved the physiological transition from autotrophy to heterotrophy in *C. zofingiensis* [28] function in *Scenedesmus* species.

5. Conclusions

Mixotrophic cultivation of *Scenedesmus* in nutrient rich wastewater can enhance biomass production, modulate lipid and carbohydrate metabolism, and remediate the wastewater in an environmentally-sustainable manner. However, economically-viable algal systems that couple biofuel production with large-scale wastewater treatment are still limited by low algal biomass productivity and high production/operational costs [128]. While this literature review confirmed the ability of *Scenedesmus* sp. to grow in organic wastewater of different sources, and simultaneously remove unidentified inorganic nitrogen and phosphorus compounds, the effect of other organic substrates on the enhancement of *Scenedesmus* sp. biomass and lipid productivity should be studied in more detail. Furthermore, there are known differences in the regulation of carbon metabolism in evolutionarily-diverse microalgal classes, as well as within different *Scenedesmus* species [129]. More genomic information and the molecular and biochemical characterization of the key metabolic pathways would offer great advantages for the production and optimization of biofuel and/or other biomaterials. Different organic carbon and organic and mineral nitrogen substrates, easily acquired from domestic and agro-industrial wastewater sources, should also be tested as nutrient sources. Furthermore, genomic and transcriptomic studies have provided some insight into the basic carbon metabolism of *Scenedesmus* species. Expansion of these systems biology approaches are needed to inform genetic engineering strategies, and to fully understand carbon partitioning and the physiological conditions that maximize productivity and nutrient removal.

Author contributions

JM and SS initially planned the outline and wrote the review, JP and SS contributed to the collection of references and reviewed and edited the text.

Declaration of competing interest

The authors declare that there is no conflict of financial or personal interests.

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Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights applicable to this study.

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