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Nutrients recovery and recycling in algae processing for biofuels production

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ABSTRACT

The supply of nutrients is a great issue to a sustainable scale-up of microalgal biofuels production, as these photosynthetic microorganisms require large amounts of N, P and other micronutrients to grow, which turns into high fertilizers demand. Additionally, recovery and reuse of nutrients (particularly N & P) are a must to reduce the non-point pollution emanating from their release into water or air during the downstream processing steps to biofuels or bioproducts. In the recent years, strong research efforts have been paid for developing nutrient recovery and recycling techniques, in order to reduce the net amount of fertilizers required. One possibility is exploiting nutrients from waste streams, such as wastewaters, while others focus on the recovery of N and P from the non-fuel fraction of the produced microalgal biomass, which is then recycled to the cultivation system, in a closed-loop perspective. In both cases, the presence of possible contaminants as well as nutrients bioavailability can impact the biomass productivity compared to standard synthetic media. Although the nutrients recovery and reuse has been in the forefront for a few years, there are no review publications available yet. In this paper, state-of-the art studies on nutrients recovery and recycling methods in microalgae processing from the last decade are reviewed. The study focuses on the different N and P recovery methods and yields, as well as on their subsequent use in algal cultivation and impact on algae productivity. Possible bioproducts exploitation is considered, and perspectives of closed-loop material balances on a large-scale are eventually provided.

1. Introduction

Microalgae are photosynthetic organisms able to produce numerous valuable compounds, such as fatty acids, proteins, pigments, and polysaccharides. Among all these, algal biomass is identified as a promising feedstock for the production of renewable liquid fuels and bioproducts thanks to its high growth rate, biochemical composition, and oil content compared to conventional energy crops [1,2].

Microalgae cultivation stands out over terrestrial crops mainly because they do not require arable land hence not directly competing with food production. Despite these acknowledged advantages, first process assessments often neglected the nutrients requirement to achieve significant biomass and biofuels productions on a large scale. In fact, microalgal biomass contains about three times the amount of nutrients compared to terrestrial plants [3], so that competition between energy (i.e. fuels), bio-products, and food production might actually be shifted from land to fertilizers issues.

Only in the last decade the problem of nutrients demand in industrial microalgae cultivation became a matter of concern in the scientific community, with special concern to nitrogen and phosphorus [3]. Inorganic nitrogen compounds are produced via the Haber-Bosch process, which involves H_2 derived from fossil sources as a reactant, together with high temperature and pressure, resulting in elevated process energy duties, and CO_2 emissions [4]. Phosphorus, on the other hand, is derived from phosphate mines, already largely exploited for agricultural crops. Recent studies showed that current rates of mined phosphorus utilization for food production are not sustainable, and phosphate reserves are expected to be depleted in the next 50–100 years [4–6].

Based on the elemental composition of microalgal biomass, and assuming 100% uptake, it is estimated that roughly 40–90 kg of N and 3–15 kg of P are required to produce 1 t of algae [6–8]. Simple material balances and resources assessments allow understanding that, if significant displacement of petroleum-derived fuels is to be achieved, these amounts cannot be sustainably met by fertilizers supply. For example, the production of 19 billion liters per year of algal oil-based fuels (roughly 25% of the target established by the United States Energy Independence and Security Act for 2022), would require 41–56% and 32–49% of N and P_2O_5 fertilizers world surplus, respectively [6]. This would likely affect fertilizers market prices, further lowering the economics of algal biofuels production. Moreover, considering that research on renewable fuels is driven by the need of reducing carbon

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dioxide emissions in the atmosphere, such a high fertilizers consumption might be counterproductive in this regard, due to the release of CO_2 in their production process [9].

Therefore it is quite clear that N and P need to be recovered from alternative sources, e.g. by exploiting wastewaters or by recycling process streams. In particular, since the oil fraction of microalgae contains only little amounts of these elements, N and P could be recovered from the biomass and recycled for further production, reducing the net fertilizers input. In the past decade, and especially in the second half of it, intense research has been focused on the investigation of possible techniques to achieve this goal.

This review paper aims at providing a comprehensive analysis and comparison of nutrients (mainly N and P) recovery and recycling methods developed in microalgae processing so far, as well as at understanding how the different recycled media affect the biomass productivity. Perspectives of material balances for large-scale applications are eventually discussed.

2. Nutrients requirement in algal cultivation

Microalgae require specific amounts of essential macro- and micronutrients to grow. Carbon, nitrogen and phosphorus are the important macronutrients, [10], which need to be supplied to the culture in bioavailable forms for an efficient uptake. Concerning C, microalgae as photosynthetic microorganisms mostly uptake the inorganic form of CO_2, dissolved in the medium. Several studies [11–13] have successfully evaluated the possibility of supplying inorganic C also in the form of soluble bicarbonate, exploiting the equilibrium of carbon ions in solution [3]. In addition, some microalgae are able to uptake organic molecules (e.g., glucose, acetate, glycerol), as a source of both carbon and energy. However, even though mixotrophic cultivation usually results in higher growth rates, the cost of organic substrates makes it an impracticable choice for large-scale biofuels production. Although C supply is certainly of great importance in microalgae cultivation, it is not the focus point of this review.

Nitrogen, which is essential for amino acids and proteins synthesis, is commonly taken up in the inorganic forms of NO_3^- or NH_4^+ [3]. The latter one, which is the inorganic N form prevailing in most waste streams, is potentially the preferred source by microalgae as, being the most reduced form, it requires less energy to be assimilated. Nonetheless, care must be paid when supplying ammonium to the cultivation medium, as in solution it is in chemical equilibrium with free ammonia, which is toxic on microalgae cells above a concentration of about 2 mM [3,14]. This drawback can indeed be controlled by either regulating the pH and/or using proper concentrations. In addition to inorganic forms, some microalgal species are able to assimilate organic nitrogen molecules. Among the most common ones is urea, but a few strains are also reported to be capable of up-taking simple amino acids [15,16].

Phosphorus, on the other hand, is mainly taken up by microalgal cells from the medium in the form of orthophosphates, while other inorganic or organic forms of P generally require to be first mineralized and converted to orthophosphates in order to be assimilated [3].

Besides C, N and P, microalgae require the presence of several other trace nutrients in the cultivation medium, such as K, Mg, S (as SO_4^{2-}), Ca and Fe, among others. Despite the small quantities required, these micronutrients are essential components of the biomass.

Standard cultivation media are formulated so that all the necessary nutrients are present in adequate amounts and ratios (see Table 1), ensuring no limitation to growth. These formulations are usually based on the Redfield ratio for phytoplankton, which considers C.N:P proportions of 106:16:1 on molar basis [17]. However, evidence has shown that microalgae composition can diverge from this ratio, and it can adapt to the environmental composition, according to nutrient availability [18]. For example some microalgae, when cultivated in a phosphorus-rich medium, tend to accumulate the excess P as intracellular polyphosphate reserves, for later use in case the medium becomes P-depleted. This phenomenon is known as luxury uptake [19], and should be avoided to maximize P utilization. In addition to the medium composition, nutrients uptake also depends on cultivation conditions, such as light intensity and, in continuous cultures, dilution rate (i.e., specific growth rate) [20,21]. As a result, the uptake of nutrients in microalgal cultivation is indeed a complex phenomenon, so that the actual amounts required to achieve high productivities are even greater than those predicted by most life cycle assessment (LCA) analyses, which consider 100% uptake efficiencies [4,7,22,23]. Therefore, it appears even more important to develop nutrients recycling technologies.

3. Seawater and wastewaters as nutrient sources

The use of alternative water sources in place of freshwater for large-scale algal cultivation has been largely encouraged. This would be beneficial both in terms of water footprint (the life-cycle usage of freshwater would be greatly reduced [8]) as well as of macro and micronutrients supply. Fig. 1 shows different nutrients-rich sources that could be exploited for algal cultivation.

Seawater contains excess amounts of most of the micronutrients required for algal growth, especially potassium, but also magnesium and sulfur. In addition, little amounts of nitrogen and phosphorus, as well as CO_2 absorbed from the atmosphere, are dissolved in the marine water environment [8,24,29,30]. Clearly, the use of seawater is limited to marine microalgae (such as *Nannochloropsis* sp. *Tetraselmis* sp.), even though a medium containing 10% of seawater has been proposed for inexpensive cultivation on freshwater algae and cyanobacteria [31]. In any case, the concentration of N and P in saline water is not sufficient to sustain significant algal growth, so it must be increased by a suitable technique in order to achieve substantial biomass productivities.

Wastewaters, on the other hand, are generally rich in N and P. In fact, the possibility of growing algae in wastewaters has gained a lot of interest because of the double advantage of simultaneously treating polluted effluents and producing valuable biomass [3]. Depending on the source, wastewaters have different compositions and characteristics, which determine their suitability for microalgae cultivation and the resulting productivity, as detailed here below.

3.1. Municipal wastewaters

Urban wastewaters have been widely investigated as viable nutrients sources for microalgae growth [26,32–34]. Although nutrient concentrations in municipal wastewaters depend on the stage of the depuration process (primary or secondary treatment), microalgae have been shown to efficiently uptake N (mainly present as NH_4^+, with little amounts of NO_3^- and NO_2^-) and P from this source, both in batch and in continuous cultivation [25,35]. Remarkable values of specific growth rates (about 0.7 d^{-1} and 1 d^{-1}) are reported for *Chlorella protothecoides* [32] and *Scenedesmus obliquus* [25], respectively. However, the final biomass production is limited by the relatively low nutrients concentrations: for N it typically ranges between 20 and 40 mg L^{-1}, for P between 3 and 10 mg L^{-1} [4,6,9,25,32], values that would allow reaching biomass concentrations not more than 0.5 g L^{-1}. Other studies exploited the effluents coming from the anaerobic digestion of either municipal wastewater [36] or of the activated sludge produced from municipal wastewater treatment [37], to efficiently cultivate the marine alga *Nannochloropsis*. These streams are more concentrated compared to the starting wastewaters, so that higher biomass concentrations can be reached, even though proper dilutions are needed to avoid inhibition. Regardless the treatment process stream considered, the amount of all municipal wastewaters available could only give a small contribution (1–5%) to the total N and P requirements necessary to satisfy the current transportation fuels demand of a large city [4,6,9].

Table 1
Composition of some common standard freshwater and seawater cultivation media.

Freshwater				Seawater			
BG11		**Bald's Basal Medium (BBM)**		**F/2**		**Artificial Seawater Medium (ASM)**	
Component	*Conc. mM*	*Component*	*Conc. mM*	*Component*	*Conc. mM*	*Component*	*Conc. mM*
$NaNO_3$	17.6	$NaNO_3$	2.94	$NaNO_3$	0.88	$NaNO_3$	11.8
K_2HPO_4	0.23	K_2HPO_4	0.43	$NaH_2PO_4{\cdot}2H_2O$	0.036	K_2HPO_4	0.37
$MgSO_4{\cdot}7H_2O$	0.3	$MgSO_4{\cdot}7H_2O$	0.3	Thiamin	0.335 (ppm)	$MgSO_4{\cdot}7H_2O$	10.5
$CaCl_2{\cdot}2H_2O$	0.24	$CaCl_2{\cdot}2H_2O$	0.17	Biotin	0.025 (ppm)	$CaCl_2{\cdot}2H_2O$	2
Citric Acid	0.031	KH_2PO_4	1.29	Vitamin B12	0.135 (ppm)	Vitamin B12	0.135 (ppm)
Ferric Ammonium Citrate	0.021	$FeCl_3{\cdot}6H_2O$	$2.16{\cdot}10^{-3}$	$Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$	$11.7{\cdot}10^{-6}$	$FeCl_3{\cdot}6H_2O$	$1.8{\cdot}10^{-3}$
$Na_2EDTA{\cdot}2H_2O$	0.0027	$Na_2EDTA{\cdot}2H_2O$	0.012	$Na_2EDTA{\cdot}2H_2O$	$0.0117{\cdot}10^{-3}$	$Na_2EDTA{\cdot}2H_2O$	0.027
Na_2CO_3	0.19	NaCl	0.43	Sea Salts	33 (ppm)	NaCl	$0.31{\cdot}10^{3}$
H_3BO_3	0.046					H_3BO_3	0.184
$MnCl_2{\cdot}4H_2O$	0.009	$MnCl_2{\cdot}4H_2O$	$1.26{\cdot}10^{-3}$	$MnSO_4{\cdot}H_2O$	$0.9{\cdot}10^{-6}$	$MnSO_4{\cdot}H_2O$	$9.7{\cdot}10^{-3}$
$ZnSO_4{\cdot}7H_2O$	$0.77{\cdot}10^{-3}$	$ZnCl_2$	$0.22{\cdot}10^{-3}$	$ZnSO_4{\cdot}7H_2O$	$0.08{\cdot}10^{-6}$	$ZnSO_4{\cdot}7H_2O$	$0.7{\cdot}10^{-3}$
$Na_2MoO_4{\cdot}2H_2O$	0.0016	$Na_2MoO_4{\cdot}2H_2O$	$0.1{\cdot}10^{-3}$	$Na_2MoO_4{\cdot}2H_2O$	$0.03{\cdot}10^{-6}$	KCl	8
$CuSO_4{\cdot}5H_2O$	$0.3{\cdot}10^{-3}$			$CuCl_2{\cdot}2H_2O$	$0.04{\cdot}10^{-6}$	NH_4Cl	0.5
$Co(NO_3)_2{\cdot}6H_2O$	$0.17{\cdot}10^{-3}$	$CoCl_2{\cdot}6H_2O$	$0.05{\cdot}10^{-3}$	$CoSO_4{\cdot}7H_2O$	$0.05{\cdot}10^{-6}$	$CoCl_2{\cdot}6H_2O$	$0.2{\cdot}10^{-3}$

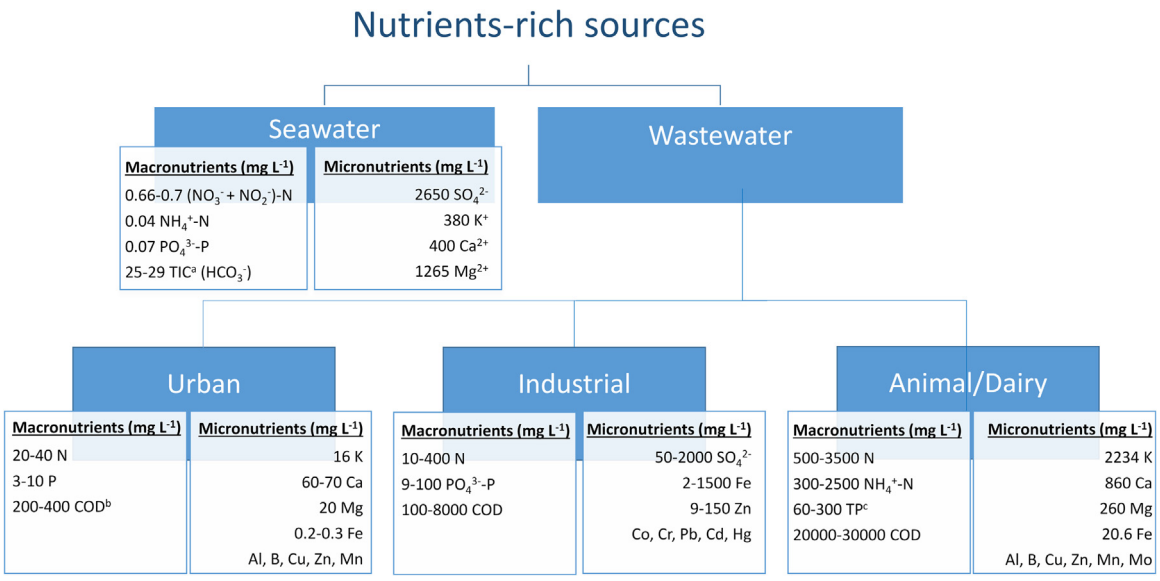

Fig. 1. Nutrients-rich water sources for algal cultivation [6,24–28], [a]Total Inorganic Carbon, [b]Chemical Oxygen Demand, [c]Total Phosphorus.

3.2. Agro-industrial wastewaters

Industrial processes generate large amounts of polluted, nutrients rich waters. Depending on the industry sector, these wastewaters have different components, which can include heavy metals. Studies of microalgal growth have been successfully performed on wastewaters generated by leather processing, textile and carpet industries, among others [26]. Besides these, the agro-food industry sector represents the major source of effluents rich in organic compounds, phosphates and nitrogen (ammonium and nitrate). For example, brewery effluents have shown to be a good substrate and nutrient medium for microalgal growth [27,38], achieving lipid productivities as high as 60 mg L^{-1} d^{-1}. Also the dairy industry is quite interesting in this respect, and efficient mixotrophic algal growth has been verified using cheese whey permeate (40% v/v, obtaining final biomass concentrations of 4.9 g L^{-1} and lipid productivities of roughly 40 mg L^{-1} d^{-1} with the alga *S. obliquus*) [39]. Efficient nutrient uptake and removal was also shown in treated and untreated dairy industry wastewaters at various dilutions.

3.3. Animal wastewaters

Large amounts of wastewaters are generated from livestock farms. When processed through anaerobic digesters for biogas production, animal manure digestates become a suitable nutrients source for algal growth, which is an interesting alternative to currently employed land disposal. They generally have high concentrations of N (as NH_4^+) and P, as well as high organic loads, so that severe dilutions are necessary in order to prevent inhibitory effects due to turbidity as well as excessive ammonia concentrations. Recovery of nutrients by microalgae has been extensively investigated for different anaerobically digested animal wastes, such as poultry, swine, cow and dairy manures [34,40,41], showing positive results in terms of biomass and lipids productions. Indeed, animal-derived wastewaters represent the major potential contribution to total N and P supply for algal cultivation, possibly able to reduce significantly the demand of synthetic fertilizers [6].

3.4. Concluding remarks

In general, wastewaters resulting from different processes have shown to be good nutrients sources for microalgal growth. Because of potential contamination by external bacteria, resilient species such as *Chlorella* and *Scenedesmus* are normally employed when using these media, which however ensure good growth performances. Nonetheless, wastewaters alone are not capable of meeting the total N and P

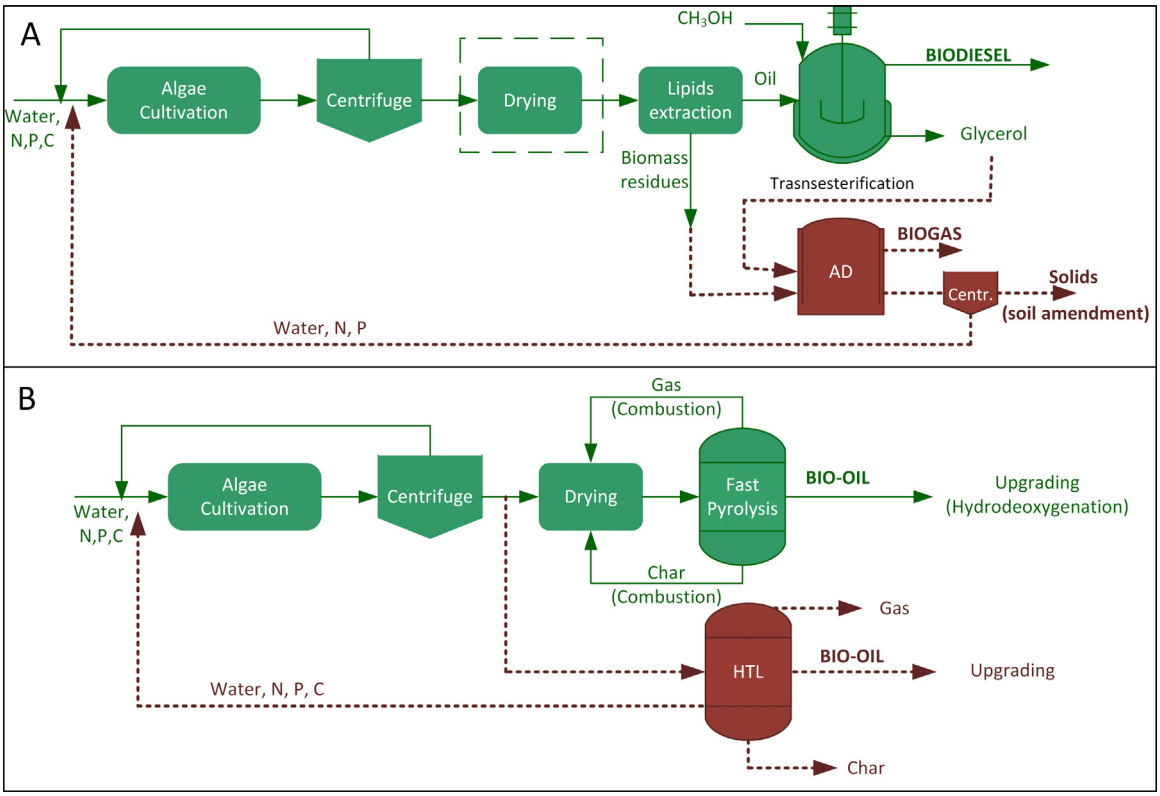

Fig. 2. Block flow diagram of microalgae liquid biofuels production processes, and their integration/improvement with nutrients recycling.

requirements to achieve significant production of algal biofuels [6,8]. In addition, they would need to be collected and transported to the algal production site, adding further logistical and economic burdens. Therefore, internal nutrients recovery and recycling strategies appear as a necessary and possibly better option to lower synthetic fertilizers input to the cultivation system.

4. Conventional algae processing methods

The traditional route for conversion of microalgae into liquid fuels is the production of biodiesel via transesterification of the lipid fraction contained in the biomass (Fig. 2A). Such a process requires harvesting and concentration of the biomass after cultivation, followed by lipids extraction. Conventional extraction methods involve the use of organic solvents, such as hexane, chloroform (alone or in a mixture with methanol), isopropanol, among others [42]. However, the algal biomass needs to be dried up to a moisture content of 10% or less, which results in high energy inputs and associated costs [2]. Recently, wet lipid extraction techniques are proposed to mitigate the costs associated with drying [43].

Once extracted, triglycerides undergo transesterification to produce Fatty Acid Methyl Esters (FAME) and glycerol, which is a massive by-product of this reaction [1].

As an alternative to the traditional transesterification process, a number of thermochemical conversion methods have been proposed. For instance, pyrolysis is carried out in the absence of oxygen/air at temperatures between 350 °C and 550 °C, resulting in the production of bio-oil (up to 80%), together with charcoal and a gaseous phase (Fig. 2B). Such bio-oil needs to be subsequently upgraded by hydrodeoxygenation to reach properties comparable to those of crude oil. Moreover, pyrolysis requires the algal biomass to be dried, leading to unfavorable energy balances, which give little hope for industrial applications. To save the drying energy duty, hydrothermal treatments (HTT) carried out on the wet biomass (Fig. 2B, see Section 5.2) have received wide attention as viable processes for conversion of microalgae into biofuels.

4.1. Advanced algal systems

In the most recent Multi-Year Program Plan (March 2016) [44], the Bioenergy Technology Office of the U.S. Department of Energy identified two possible routes for microalgal biomass conversion into biofuels (or into their intermediates, i.e. lipids/bio-oil). The Combined Algae Processing (CAP) pathway, based on biomass fractionation, comprises fermentation of the sugar fraction to produce bioethanol, followed by wet lipids extraction and conversion to biodiesel as described previously. This way the production of biofuels is maximized, but such an approach is clearly sensitive to the biochemical composition of the microalgae biomass, which therefore should be accurately controlled. The alternative pathway is hydrothermal liquefaction (HTL), which is instead carried out on the whole biomass, and is less influenced by the quality of the algal feedstock.

In both cases, the main costs are associated with the biomass production step rather than with the conversion pathway, highlighting how substantial improvement is required in the cultivation system in order to lower the minimum biofuels selling prices. In addition, co-products exploitation and recycling of resources are deemed essential for the sustainability and scalability of these processes.

5. Nutrients recovery methods

Regardless the algae-to-fuels pathway, the integration with nutrients recycling from the residual biomass is necessary to obtain a sustainable production of algal biomass. Different processes and strategies have been developed to this aim, which can be mainly distinguished between biological (i.e. anaerobic digestion) and hydrothermal treatments.

5.1. Anaerobic digestion

Anaerobic digestion (AD) is a well-known and a commercially developed technology that involves the biological conversion of complex organic matter into biogas (i.e. a mixture of mainly CH_4 and CO_2, with minor percentages of H_2S, NH_3, H_2O) through a suitable sludge inoculum [45].

Anaerobic digestion of microalgae has been proven feasible, using both whole biomass (WA) and the residue remaining after lipids extraction (LEA), with methane yields ranging from 100 to 550 mL gVS^{-1} [46–50]. This biogas could be used for the generation of electricity or heat to be exploited within the process itself, potentially increasing the energy revenues [46,51], or upgraded to biomethane as transportation fuel [52]. At the same time, the nutrients contained in the biomass are mineralized and solubilized into the liquid effluent, i.e. the digestate. Compared to other types of biomass, however, AD of microalgae has some intrinsic challenges, namely the low degradability of the cell wall, which acts as a protection of the intracellular organic matter from bacteria, and the low C/N ratio due to the high protein content, which might lead to excessive production of ammonia, resulting in toxicity effects [46,51].

Therefore, it is preferable to carry out AD on microalgae residues (Fig. 2A). In addition to exploiting the lipid fraction for the production of biodiesel, the extraction process disrupts the cell walls and increases the solubilization of the organic matter, resulting in higher biodegradability of the residual biomass [49]. Clearly, appropriate solvents must be used for lipids extraction, to avoid hindering anaerobic bacteria in the digestion process [53]. Bohutskyi et al. [48] reported that the integrated process coupling conversion of lipids to biodiesel with biomethane production from the spent biomass generates 100% and 40% more energy than biodiesel or biomethane production alone, respectively. Moreover, the glycerol obtained as a by-product of the transesterification process could be used as co-digestion substrate to increase the C/N ratio in the digester [54].

Table 2 summarizes the results obtained by various authors in terms of methane yield and nutrients recovery from the anaerobic digestion of either raw, lipid-extracted, or pre-treated microalgae biomass. According to most authors, when AD is carried out on lipid-extracted biomass the biodegradability is increased (ranging between 35% and 55%), as well as the fractions of N and P recovered. Other biomass pretreatments, such as thermal [48], sonication [50], enzymatic hydrolysis [48] or high pressure thermal hydrolysis (HPTH) [55], tend to enhance the methane yields and nutrients recovery, but require increased operational costs. The fraction of N and P recovered in the liquid digestate depends on the duration of the AD process (i.e. the Hydraulic Retention Time, HRT) as well as on the Organic Loading Rate (OLR), and varies between 30% and 70% for nitrogen, with an average of about 60%, and between 3% to more than 90% for phosphorus. The lowest N recovery yield was found by Bohutskyi et al. [47,48] in semi-continuous AD of both *Auxenochlorella protothecoides* and *Nannochloropsis salina*, explained by either volatilization or poor protein degradation. Concerning P, Keymer et al. [55] and Sforza et al. [56] report that, while nitrogen recovery appears somehow correlated to the biodegradability, other phenomena related to phosphorus solubility limit the recovery of this nutrient. Depending on the pH value, P tends to precipitate forming insoluble salts (e.g. struvite, calcium phosphate, iron and aluminum phosphates [57]), so that chemical post-treatment might be necessary to increase the solubilized portion. Bohutskyi et al. [47,48] also characterized the recovery of other micronutrients (e.g. Fe, S, Ca, Co, Mn, Cu, Zn, etc.), assessing that between 10% and 50% of

Table 2
Summary of methane yield and nutrients recovery from anaerobic digestion of microalgae reported in scientific literature.

Anaerobic digestion							
Microalgae species	**Pre- treatment**	**Experimental conditions**	**Methane yield**	**Nutrient recovery**		**Digestate concentration (mg L^{-1})**	
			(mL gVS^{-1})	**N**	**P**	**N**	**P**
Chroococcus sp. [58]	WA	36 °C, 45 d S/I = 0.3	317 ± 2	69.6%	*na*	196.63 ± 2.37 NH_4-N 46.34 ± 1.48 NO_3-N	45.2 ± 2.16 TP
Chlorella sorokiniana [59]	WA	35 °C, 74 d S/I = 0.5	*na*	59%	89%	637 ± 20 NH_4-N	90 ± 2.3 PO_4-P
Chlorella sorokiniana [50]	WA	30 °C, 42 d	298	48.1%	87.7%	90 NH_4-N	30 TP
	LEA	30 °C, 42 d	253	61.5%	93.6%	120 NH_4-N	30 TP
	Sonication, WA	30 °C, 42 d	388	77.4%	99.4%	150 NH_4-N	30 TP
Chlorella vulgaris [56]	LEA	35 °C, 41 d S/I = 0.5	150 ± 15	*na*	2.5–41%	524 NH_4-N	0.88–14 PO_4-P
Auxenochlorella protothecoides [47]	LEA	35 °C, 20–40 d	180–250	25–40%	30–60%	200–500 TN	100–500 TP
Nannochloropsis salina [48]	WA	35 °C, 20 d	240 ± 20	*na*	*na*	*na*	*na*
	Thermal, WA	35 °C, 20 d	310	*na*	*na*	*na*	*na*
	LEA	35 °C, 20 d	220 ± 20 [1]	22–30%	60–70%	300–1100 TN 180–500 NH_4-N	200–650 TP 200–620 PO_4-P
	Enzymatic, LEA	35 °C, 20 d	250	30%	70%	1200 TN 500 NH_4-N	700 TP 650 PO_4-P
Scenedesmus sp. [55]	WA	38 °C, 35 d S/I = 0.5	180 ± 10	43%	25%	*na*	*na*
	HPTH, WA	38 °C, 35 d S/I = 0.5	330 ± 10	56%	20%	*na*	*na*
	LEA	38 °C, 35 d S/I = 0.5	240 ± 10	56%	33%	*na*	*na*
	HPTH, LEA	38 °C, 35 d S/I = 0.5	380 ± 10	63%	40%	*na*	*na*

WA = Whole algae.
LEA = Lipid-extracted algae.
HPTH = High Pressure Thernal Hydrolysis.
S/I = Substrate/Inoculum ratio (VS basis).
TN = Total Nitrogen.
TP = Total Phosphorus.
na = not available.

these elements were solubilized in the liquid digestate.

5.2. Hydrothermal treatments

Hydrothermal treatments have gained a lot of interest in the conversion of algal biomass, thanks to the possibility of processing wet microalgae directly (5–20% solid content), avoiding the energy-costly drying step. They exploit the properties of water under sub/supercritical conditions, as under high temperatures and pressures chemical and physical properties of water change, so that non-polar organic compounds become increasingly miscible in it. These tunable physiochemical properties allow targeting the reaction toward the production of solid, liquid or gaseous fuels by simply varying the temperature and pressure, together with the residence time [60,61]. Hence, depending on the operating conditions, different processes can be distinguished. All of them generate an aqueous phase (AP) rich in nutrients, which therefore has the potential to be recycled as cultivation medium for algal growth.

5.2.1. Hydrothermal Gasification (HTG)

Also known as *Supercritical Water Gasification*, this process is conducted above the water critical point (374 °C and 22.1 MPa), with temperatures ranging between 400 and 700 °C and pressures of 25–30 MPa [61,62]. In such conditions, the algal feed is converted into energy-rich gaseous products (CH_4 and H_2) together with CO, CO_2 and C_2-C_4 compounds, with HHV up to 36 MJ m^{-3} [63], and carbon gasification efficiencies as high as 98% [64,65].

Some authors [64–66] have characterized the recovery of nutrients from HTG of microalgae (Table 3). The AP generated from the process is rich in N, a large fraction of which (40–50%) is composed by NH_4-N, with overall recoveries ranging between 33% and 57% of the initial biomass content. Regarding P, Patzelt et al. [64,67] report very low recoveries (~ 1%) and concentrations of 1–3 mg L^{-1} in the AP. Although these authors do not explain such a result, it is likely that precipitation of P inorganic salts occurs, owing to the marked drop of their solubility in supercritical water. Besides N and P, a number of other nutrients (K, Mg, Ca) and trace metals, are also extracted in the aqueous phase.

Despite the promising aspects of HTG, this technology is associated with unsustainably high energetic and capital costs due to severe operating conditions, which make it rather unfavorable for large-scale applications.

5.2.2. Hydrothermal Liquefaction (HTL)

Under moderate temperatures, ranging between 250 and 370 °C, and 10–22 MPa (sub-critical water conditions), HTL converts the wet algal biomass into a liquid biocrude as main product, together with a solid residue, an aqueous phase (AP) and a gaseous fraction. The typical residence times vary between 10 and 60 min. HTL is considered the most promising treatment option for liquid fuels production from algal biomass, and extensive research work has been carried out in this field. On average, the HHV of the biocrude produced by HTL ranges between 30 and 50 MJ kg^{-1} [60]. Some issues connected with HTL are related to the separation of the organic liquid product from the aqueous phase, together with the fact that, despite the high heating value, the biocrude produced through HTL needs significant upgrading before it can be used as a transportation fuel because of high oxygen, nitrogen and sulfur contents, together with acidity and viscosity issues.

The AP generated by the process contains significant amounts of nutrients as well as soluble organics. Recoveries of up to 80% are reported for both N and P (Table 3), even though these values strongly depend on the operating conditions as well as on the microalgal substrate. For example, under higher temperatures more of the nitrogen is found in the biocrude fraction, even though the percentage of inorganic ammonium over organic N compounds tends to increase (up to 80%), because of enhanced decomposition. Besides, high temperatures favor P precipitation, so that its recovery in the AP is reduced [68]. On the other hand, phosphorus recovery is also highly dependent on the microalgal species, or more specifically on the composition of other inorganic compounds present in the feedstock: for example, López-Barreiro et al. [69] found similar nitrogen, but very different P recoveries from HTL of *Scenedesmus almeriensis* (< 1%) and *Nannochloropsis gaditana* (up to 63%) under the same operating conditions. The authors also report that neither Mg^{2+} or Ca^{2+} are found in the AP of both species because of precipitation, suggesting that the low P recovery obtained with *S. almeriensis* might be related to the higher concentration of these elements in the starting feedstock. Together with high concentrations of N (mainly ammonia) and P, the AP is also rich in soluble organic compounds. Some authors [68,70,71] suggest integrating Supercritical Water Gasification carried out on the AP from HTL in order to gain more energy and fuel production from this carbon. Elliott [68] reports that in this way more than 99% of the COD contained in the AP could be converted to a gas fuel product. This step could also be beneficial in view of the subsequent recycling of the AP for algal cultivation, as the load of potential toxic organic compounds is reduced, while the organic N compounds are further degraded to more bio-available ammonium [70].

5.2.3. Hydrothermal Carbonization (HTC)

HTC is characterized by the mildest operating conditions, i.e. low to moderate temperatures (120–250 °C), and pressures around 2 MPa. The residence time is generally quite long, in the order of hours, and the main product is a solid with coal/char properties. Solid yields of up to 60% are reported, with a HHV of around 30 MJ kg^{-1}.

In addition, most of the lipids are retained in this hydrochar, and can be later recovered by a simple solvent extraction [72,73]. While the majority of C ends up in the solid char, significant nutrients fractions (50–80% of N and 60–100% of P) are recovered in the AP (Table 3), generally increasing with temperature and/or reaction time. Due to the mild temperatures used, most of the solubilized nitrogen (about 90%) is present in organic form. Yao et al. [74] report that only a small fraction (~ 22%) of the organic N is composed by amino acids, the rest being mostly polypeptides derived from proteins decomposition and heterocyclic compounds formed through Maillard-type reactions.

On the other hand, NH_4-N represents a small amount of the total N recovered, ranging between 10% and 20%, with the highest value obtained under longer reaction time and higher temperature [88].

5.2.4. Flash Hydrolysis (FH)

Another interesting hydrothermal process is Flash Hydrolysis, which is carried out under sub-critical water conditions, in a temperature range similar to that of HTL (250–350 °C), but is characterized by extremely short residence times (< 10 s). Similarly to HTC, the main products of the process, are a lipid-rich solid fraction and an aqueous phase, in which hydrophilic oligopeptides and amino acids are extracted as a result of proteins hydrolysis. The solid product, with yields ranging between 20% and 50% of mass recovered, is enriched in carbon (up to 67%) as well as in lipids (up to 74% w/w) content [85,87], and can be considered as a biofuels intermediate (BI). Teymouri et al. [89] showed that the FAME profile of the lipids preserved in the BI is consistent with that of the starting microalga.

Good amounts of nitrogen and phosphorus are extracted in the AP, with recoveries ranging between 50% and 80% (Table 3), together with other inorganic elements (e.g. S, K, Mg, Ca, etc.) [85,89]. While the P present in the aqueous hydrolyzate is mainly in the form of orthophosphates, due to the short residence time only 10–15% of the recovered nitrogen is inorganic ammonium, the majority being available as protein building blocks, with glycine and arginine found in significant amounts [87]. The formation of undesired ring-type Maillard compounds is instead minimized by the FH process.

A great advantage of this process is that the short residence time allows easily working in continuous-flow systems, with precise control

Table 3
Summary of fuel product yield and nutrients recovery from hydrothermal treatments of microalgae reported in scientific literature.

HTG						
Microalgae species	**Operating conditions**	**Gas yield**	**Nutrient recovery**		**AP concentration (mg L^{-1})**	
			N	**P**	**N**	**P**
Chlorella vulgaris [65]	500 °C, 30 min	11 mol_{H2}/g	33–49%	4–47%	1830–2670 TN 750–1280 NH_4-N 25–46 NO_3-N	17–221 PO_4-P
Spirulina platensis [65]	500 °C, 30 min	11 mol_{H2}/g	27–57%	5–24%	2020–4290 TN 900–1780 NH_4-N 13–19 NO_3-N	28–128 PO_4-P
Acutodesmus obliquus [64,67]	600–650 °C, 15–30 min	10 L h^{-1}[a]	56.8%	1.3%	2230–3200 TN	1.1–3 TP
HTL						
Microalgae species	**Operating conditions**	**Biocrude yield**	**Nutrient recovery**		**AP concentration (mg L^{-1})**	
			N	**P**	**N**	**P**
Desmodesmus sp. [75]	300 °C, 5 min	40%	*Na*	*na*	1964 Organic N 2012 NH_4-N 70 NO_3-N	160 PO_4-P
Nannochloropsis sp. [76]	250–400 °C, 10–90 min	35–50%	67–84%	38–85%	1000–14000 TN	*na*
Phaeodactylum tricornutum [71]	350 °C	31%	87.5%	100%	6500 NH_4-N 248 NO_3-N	783 PO_4-P
Chlamydomonas reinhardtii [77]	220–310 °C, 60 min	59–67%	13–51%	41%	1866–7311 NH_4-N	1729 PO_4-P
Spirulina platensis [78]	350 °C, 60 min	40%	75%	28.3%	16,200 TN	791 TP
Aurantiochytrium limacinum [79] (lipid-extracted)	200 °C, 10 min	3.1%	73%	70.3%	588 TN 48 NH_4-N	77 PO_4-P
Aurantiochytrium limacinum [79] (lipid-extracted)	250 °C, 60 min	17%	100%	82.5%	937 TN 234 NH_4-N	74 PO_4-P
Scenedesmus almeriensis [69,70]	300–375 °C, 5–15 min	52–62%	60–67%	0.7%	5296 TN 3514 NH_4-N	114 PO_4-P
Nannochloropsis gaditana [69,70]	300–375 °C, 5–15 min	60–63%	55–58%	50–63%	4901 TN 4509 NH_4-N	3393 PO_4-P
Chlorella vulgaris [80]	300 °C, 60 min	46.6%	74%	*na*	6636 TN 4412 NH_4-N 74 NO_3-N	1015 PO_4-P
Scenedesmus dimorphus [80]	350 °C, 60 min	27.1%	35%	*na*	3139 TN 4106 NH_4-N 43 NO_3-N	480 PO_4-P
Spirulina platensis [80]	300 °C, 60 min	35.5%	66.5%	*na*	8136 TN 4896 NH_4-N 44 NO_3-N	705 PO_4-P
Chlorogoeopsis fritschii [80]	300 °C, 60 min	38.6%	70%	*na*	5636 TN 3693 NH_4-N 115 NO_3-N	91 PO_4-P
HTC						
Microalgae species	**Operating conditions**	**Solid char yield**	**Nutrient recovery**		**AP concentration (mg L^{-1})**	
			N	**P**	**N**	**P**
Chlamydomonas reinhardtii [81]	200 °C, 2 h	39%	79%	*na*	*na*	*na*
Chlamydomonas reinhardtii [72]	200 °C, 2 h	31.3%	80%	100%	938 NH_4-N 14 NO_3-N	713 PO_4-P
Dunaliella salina [81]	200 °C, 3 h	36%	67%	*na*	*na*	*na*
Nannochloropsis oculata [73]	200 °C, 15 min	47%	63%	57%	3280 TN	440 TP
Arthrospira platensis [82,83] (carbohydrate-extracted)	190–210 °C, 2–4 h	*na*	78–91%	*na*	3188–5740 TN 429–691 NH_4-N	*na*
FH						
Microalgae species	**Operating conditions**	**Solid BI yield**	**Nutrient recovery**		**AP concentration (mg L^{-1})**	
			N	**P**	**N**	**P**
Nannochloropsis gaditana [84]	280 °C, 9 s	46.8%	50%	60%	767 TN	117 TP
Scenedesmus sp. [85–87]	205–325 °C, 6–9 s	52–19%	30–68%	*na*	210–556 TN (10–15% NH_4-N)	*na*

AP = Aqueous phase; BI = Biofuels Intermediate; TN = Total Nitrogen; TP = Total Phosphorus; *na* = not available.
[a] A proper gas yield value could not be retrieved, so the flow rate is reported instead.

of the reaction time and no need of prolonged heating/cooling periods, which makes it attractive for large-scale applications.

6. Nutrients recycling options

Once nutrients are recovered from the biomass, recycling them to the cultivation system to sustain algal growth and reduce the requirements of fresh fertilizers is not straightforward. Their bio-availability, utilization efficiency as well as possible toxicity effects need to be carefully assessed in order to make proper process evaluations. Hereafter, different strategies for recycling recovered nutrients are presented and discussed. The main results obtained in terms of growth/productivity when using these recycled nutrients are summarized in Table 4.

6.1. Direct nutrient recycling

The simplest way of supplying the recovered nutrients to the cultivation system is direct recycling of the aqueous stream produced by the conversion process, with the advantage of simultaneously reducing the net freshwater input.

6.1.1. Liquid digestate from AD

Even though several papers can be retrieved from the literature which address microalgal cultivation in liquid digestates obtained from AD of various types of feedstocks (Section 3), not so many reports are

Table 4
Summary of growth results from nutrients recycling in microalgal cultivation.

Microalgae species	AP from	Dilution	Cultivation mode	Growth	Specific growth rate (d^{-1})	Biomass productivity ($g\,L^{-1}\,d^{-1}$)	N Removal ($mg\,L^{-1}$)	P Removal ($mg\,L^{-1}$)	Mixotrophy
Chroococcus sp. [58]	AD	3× (water)	Batch, flasks	60% of control	*na*	0.07[a]	61	12.11	Yes
		3× (BG11)		~ control	*na*	0.12[a]	*na*	*na*	
		3× (wastewater)		~ control	*na*	0.11[a]	*na*	*na*	
Chlorella sorokiniana [57]	AD	10–20×	Batch, flasks	~ control	0.85[a]	0.06[a]	*na*	*na*	
Nannochloropsis oceanica [48]	AD	10–20×	Batch, flasks	~ control	0.46–0.77	*Na*	*na*	*na*	
Chlorella vulgaris [56]	AD	2×	Batch, Drechsel bottles, CO_2 5% v/v $+SO_4^{2-}$	~ control	2.07	0.25[a]	106	3.9	
Tetraselmis sp. [90]	AD	10–40×	Batch, Plastic bag PBR, air	Poor	0.1[a]	*Na*	*na*	*na*	
Acutodesmus obliquus [64,67]	HTG	355×/AC filtration	Batch, Tubes, CO_2 4% v/v	~ control	0.82[a]	1.3	149.4	122	
Chlorella minutissima. [78]	HTL	500×	Batch, flasks CO_2 5% v/v	50.5% of control	0.08	0.07	*na*	*na*	
Chlorella vulgaris [80]	HTL	400×	Batch, flasks, air	~ 50% of control	0.3[a]	$4{\cdot}10^{-3}$[a]	9.4	*na*	Yes
Scenedesmus dimorphus [80]	HTL	400×	Batch, flasks, air	~ 40% control	0.3[a]	$4{\cdot}10^{-3}$[a]	10.3	0.13	Yes
Spirulina platensis [80]	HTL	400×	Batch, flasks, air	~ control	*na*	0.05[a]	*na*	*na*	Yes
Chlorogloeopsis fritschii [80]	HTL	400×	Batch, flasks, air	30% more than control	*na*	0.04[a]	23.33	*na*	Yes
Desmodesmus sp. [75]	HTL	311× (water)	Batch, glass bottles, CO_2-enriched air	20% of control	0.67[a]	0.19	8	1.55	
		320× (COMBO)		~ control	1.23[a]	0.61	13	1.55	
Chlamydomonas reinhardtii [77]	HTL	140×	Batch, incubation shakers, CO_2 2% v/v	~ or higher than control	0.6	0.23[a]	*na*	*na*	Yes
Nannochloropsis gaditana [70]	HTL	300–600× (fresh medium)	Batch, flasks	~ control	0.71[a]	0.04[a]	14.8	*na*	
Phaeodactylum tricornutum [70]	HTL	300–600× (fresh medium)	Batch, flasks	25% of control	0.62[a]	0.015[a]	14.8	*na*	
Scenedesmus almetriensis [70]	HTL	300–600× (fresh medium)	Batch, flasks	33% of control	0.58[a]	0.01[a]	12.6	*na*	Yes
Chlorella vulgaris [70]	HTL	300–600× (fresh medium)	Batch, flasks	~ control	0.52[a]	0.023[a]	20.6	*na*	Yes
Chlorella vulgaris [92]	HTC	50–200×	Batch, flasks	more than control	0.42[a]	0.016–0.092	30–80	2–6	Yes
Arthrospira platensis [88]	HTC	25×	Batch, flasks	~ or higher than control	*na*	0.09[a]	7.6–31	*na*	Yes
Nannochloris/Synechocystis [73]	HTC	60×	Two-stage batch, Bubble column reactor	~ control	0.29[a]	0.15[a]	23–31	*na*	
Scenedesmus sp. [93]	FH	8–40×	Batch, plastic bottles, air	~ control	0.14[a]	0.06[a]	*na*	9.7	
	HTL	50–120×		Poor	*na*	*Na*	*na*	0	
Oocystis sp. [93]	FH	30–130×	Batch, plastic bottles, air	more than control	0.2[a]	0.05[a]	*na*	22.8	
	HTL	20–100×		30% of control	0.13[a]	0.02[a]	*na*	9.7	
Nannochloropsis gaditana [89]	FH	3×	Batch, Drechsel Bottles, CO_2 5% v/v	less than control	0.29	0.12[a]	41	6.17	
Scenedesmus obliquus [94]	FH	1.2–3 ×	Batch, Drechsel Bottles, CO_2 5% v/v	more than control	1.05	*na*	98[a]	21[a]	Yes
			Continuous, flat-panel PBR, CO_2 5% v/v	more than control	*na*	0.932	180[a]	25.8[a]	
Scenedesmus sp. [95]	FH	$MgNH_4PO_4{\cdot}H_2O$ precipitated from AP and dissolved in BG11	Batch, Glass bottles, air	~ control	0.98	0.115[a]	43.2	5.2	
			Continuous, Glass bottles, air	~ control	*na*	0.328	*na*	5.13	

na = not available.
[a] Calculated from data in the reference.

available on growth performances using digestate from microalgal biomass. In a closed-loop perspective, this aspect is indeed relevant as the composition of the biomass fed to the anaerobic digester affects the final composition and nutrients ratio in the liquid medium. Bohutskyi et al. [57] tested the growth of *Chlorella sorokiniana* in the digestate obtained from AD of a filamentous algae poly-culture. They verified that the nutrients ratios in the digestate, expressed using nitrogen as reference nutrient, were overall lower compared to those of the Bold's Basal Medium (BBM) used as control, but mostly coherent with those of *C. sorokiniana* biomass. In fact, the growth performances in the digestate resulted comparable or even superior to those obtained in BBM, when proper dilutions were applied. In particular, dilutions in the range of 5–10% were found suitable, with lower dilutions (20% digestate) resulting in growth inhibition either for toxicity or light limitation effects, while higher dilutions (1% digestate) lead to nutrients limitation. The same research group evaluated the growth performances of *Nannochloropsis oceanica* in the liquid digestate produced by AD of lipid-extracted residues of a microalga belonging to the same genus (*N. salina*) [48]. The results obtained were similar to the previous case, with growths comparable to those achieved in synthetic f/2 medium when using 5% digestate dilutions.

Prajapati et al. [58] investigated a closed-loop process involving AD of the cyanobacterium *Chroococcus* sp. aimed at biogas production, followed by recycling of the digestate supernatant to the cultivation of the same organism. The authors found that a 30% dilution of the digestate was optimal and allowed the maximum growth. However, the final biomass concentration reached was only 60% of that obtained in the control BG11 medium. Since the removal of N and P was not 100%, a limitation by some other micronutrients was supposed to occur: in fact, by diluting the digestate with BG11 or with rural wastewaters instead of tap water, the biomass production was close to that of the control.

On the other hand, Sforza et al. [56] found that the growth of *Chlorella vulgaris* in the digestate obtained from AD of the same microalgal species (after lipid extraction) was comparable to that of the control (BG11) only when external SO_4^{2-} was supplied to the medium. In fact, during the digestion, S is either lost in the biogas as H_2S, or it remains dissolved in the liquid digestate (Bohutskyi et al. report recovery values of 20–30% [47,48]), but in a reduced form, which was likely not bio-available for the microalga.

In summary, the liquid digestate obtained from AD of microalgae biomass can be effectively recycled to the cultivation system to sustain algal growth, even though suitable dilutions (which depend on the initial concentration of the biomass fed to the digester) or micronutrients integration might be necessary. It is worth noting that the applicability of this process is species-dependent, as not all microalgae are able to grow in complex substrates. For example, Erkelens et al. [90] showed that *Tetraselmis* sp. was not able to grow in the digestate obtained from the same algal species as well as in f/2 medium, even when using proper dilutions, and that the lipid content of the biomass was also reduced compared to the control.

6.1.2. Aqueous phase from hydrothermal treatments

The possibility of cultivating microalgae in the AP produced by the various hydrothermal processing routes described in Section 5.2 has been widely investigated in view of an efficient nutrients management and closed-loop recycling. The operating conditions, which may be targeted to gaseous, liquid or solid fuel products, influence the composition and concentration of the corresponding aqueous phase, which also determine its recycling potential.

Under the harsh conditions of HTG, several potential toxic substances inhibiting algal growth (mostly of aromatic kind) have been detected in the AP. Patzelt et al. [64], for example, report that no algal growth nor photosynthetic activity occurred in the AP obtained after HTG of *Acutodesmus obliquus*, unless heavily diluted (355 fold) in order to decrease the concentration of inhibitors. Alternatively, by treating the medium through activated carbon filtration, most of the undesired compounds could be removed, achieving microalgal growth and lipid productivity comparable to those obtained in control media [64,67]. A common characteristic of HTG-AP is the reduced content of phosphorus, so that while N can be efficiently recycled through this process after appropriate treatment, P and other nutrients still need to be provided from external sources.

The AP produced by HTL, on the other hand, is generally characterized by high concentrations of ammonium, phosphates, and organic carbon. These high values unavoidably require strong dilutions (200–600 fold), as both ammonia and phenolic compounds are reportedly toxic for algal cells when present in excess. Early studies conducted by Jena et al. [78] and Biller et al. [80] with different microalgae species, came to the conclusion that low dilutions of the aqueous phase result in growth inhibition due to toxicity effects, while if dilution is increased to avoid this phenomenon, a reduced biomass production is obtained compared to synthetic media, because of nutrients limitation. A work by Garcia Alba et al. [75] suggests that limitation by some other micronutrient (e.g. magnesium) might be the main cause of reduced algal growth rather than inhibitors concentration, as they verified that a 20× dilution of AP using COMBO medium allowed *Desmodesmus* sp. to grow four times as much as when diluting with just water. At the same time, these authors acknowledge that the toxic compounds might accumulate when repeated HTL-AP recycling are performed. In addition, they verified that after 5 cycles microalgal growth and productivity was still equal to that of control, even though cell morphology was reported to change in response to AP recycling [91]. Good results were obtained also by López Barreiro et al. [70], who reported the possibility of replacing up to 75% of nutrients (600× dilution) of standard medium by directly recycling HTL-AP for the cultivation of *C. vulgaris* and *N. gaditana*. On the other hand, this study highlights that the growth and recycling performances are strain-dependent, as *S. almeriensis* and *P. tricornutum* were instead not able to grow satisfactorily in the same APs.

Another interesting point related to the high organic carbon level in the AP after HTL is the possibility of exploiting this substrate for mixotrophic growth. In particular, acetate is reported as one of the main organic compounds present, and many microalgae species are able to utilize this substrate. Some authors report higher growth rates and biomass concentrations achieved in the HTL-AP when compared to the control medium for species that exhibit mixotrophic growth, such as *Chlorogoeopsis* sp. [80] and *C. reinhardtii* [77]. Aida et al. [79] in addition verified the possibility of utilizing the AP from HTL for heterotrophic growth of the microalga *Aurantiochytrium limacinum*. The capability of uptaking organic carbon sources for mixotrophic growth is strain-dependent as, for example Garcia Alba et al. [75] measured no TOC nor organic N consumption by *Desmodesmus* sp. in their experiments.

While mixotrophic growth is not essential when recycling the AP from HTL, thanks to the high amount of inorganic, bioavailable ammonium, it becomes important when considering the recycle of APs produced by HTC and FH. These APs contain most of the nitrogen (80–90%) as simple organic forms, mainly hydrolyzed proteins, as shown from the van Krevelen plots proposed by Levine et al. [73] and Kumar et al. [86], respectively. Nonetheless, a few studies report successful results when cultivating different microalgal species in these aqueous phases. *Chlorella vulgaris* [92], *Arthrospira platensis* [88], as well as a bi-culture of *Nannochloris* and *Synechocystis* sp. [73], produced more biomass and generally more lipids in the AP from HTC, compared to photoautotrophic cultivation in synthetic control media. The study of Du et al. [92] showed that *C. vulgaris* actually consumed 50–61% of the organic carbon content from the medium, as well as organic nitrogen, with P being the limiting nutrient. Correspondingly, Yao et al. assert that 30% of the nitrogen accumulated in the biomass of *A. platensis* cultivated in 25× diluted HTC-AP derived from organic N consumption. These authors also showed that biomass production tends to

decrease when using AP produced under harsher temperature and reaction time.

Similar results were obtained from cultivation experiments using the hydrolyzate produced by FH. Talbot et al. [93] were able to replace up to 50% of the P from control medium with the AP in the cultivation of *Scenedesmus* sp. and *Oocystis* sp, obtaining performances comparable with the corresponding controls. In contrast, when using the AP recovered from HTL (under the same T of 280 °C but residence time of 30 min instead of 9 s), they also verified that much lower or no growth occurred, possibly because of ammonia toxicity. The work of Barbera et al. [94] showed that the microalga *Scenedesmus obliquus* was able to grow efficiently in the hydrolyzate as the only source of nutrients, in both batch (with almost double specific growth rate) and continuous cultivation. In the latter, a steady state biomass productivity of $0.9\,g\,L^{-1}\,d^{-1}$ was obtained by optimizing the concentration of inlet AP, which contained all the necessary micronutrients as well as a suitable N:P ratio (7:1 w/w). On the other hand, the marine alga *Nannochloropsis gaditana* did not grow in the corresponding hydrolyzate as well as in the control f/2 medium [89]. These authors verified that no organic carbon was consumed from the substrate, indicating that *N.gaditana* could not grow mixotrophically, and slow release of ammonium from peptides degradation was necessary to sustain growth. Overall, it appears that HTC and FH produce aqueous phases more suitable for direct recycling compared to HTL or HTG, thanks to reduced formation of toxic heterocyclic and Maillard-type reaction compounds. This is a consequence of milder temperature (HTC) or residence time (FH) applied, which is also reflected in lower dilution needs (3–60 fold). However, microalgal species capable of up-taking simple organic nitrogen compounds (i.e. amino acids), such as *Chlorella vulgaris*, are to be employed in these cases.

6.2. Precipitation of nutrients as minerals

Direct recycling of the aqueous phases produced by different biomass conversion processes has shown promising results, especially when mixotrophic algal growth occurs thanks to organic substrates dissolved in the recycled medium. However, a number of drawbacks have been observed, such as the need of heavy dilutions (not really practicable in view of large-scale continuous-flow processes), growth hindering caused by the presence of toxic compounds, high risk of bacterial contamination because of the high organic matter, possible low bio-availability of nitrogen when non-mixotrophic algal species are employed. A possible alternative to overcome such problems is precipitating the inorganic nutrients from the AP, thus obtaining mineral salts to be recycled as fertilizers.

A process including precipitation of inorganic salts in hydrothermal algae-to-fuel conversion, suggesting their possible reuse for nutrient recycling to the cultivation system, was proposed already in 2009 (SunCHem [96]). This concept was then further investigated by Elliott et al. [97] in developing an efficient continuous-flow catalytic HTG process: given the tendency of inorganic salts to precipitate under the typical operating conditions, their separation prior to the fixed bed catalytic reactor helps in preserving the metal catalyst from deactivation. The characterization of the mineral solid products recovered by processing different algal feedstocks showed a high P content, together with S, Mg, Ca and Fe, suggesting that sulfates and phosphates were the main precipitated compounds. In addition, 3–4% of nitrogen was also recovered in the mineral fraction, together with ~ 35% of C (probably as carbonates). Velasquez et al. [71] also suggest that salt separation and subsequent gasification carried out on the AP following HTL would improve the process performances in terms of nutrients and energy management.

More recently, Barbera et al. [95] investigated the possibility of precipitating phosphates and the inorganic nitrogen (i.e. ammonium) fraction from the AP obtained by FH of *Scenedesmus* sp. in the form of magnesium ammonium phosphate MAP ($MgNH_4PO_4$), or struvite. The reported, non-optimized, nutrient removals from the liquid phase into the solids are 66% PO_4-P and 30% NH_4-N, with an overall 47% P recovery from the starting biomass. The capability of different microalgal strains to utilize this mineral as a nutrient source has been reported for the freshwater *Chlorella vulgaris* [98] as well as for the marine *Nannochloropsis salina* and *Phaeodactylum tricornutum* [99]. In this study it was verified that microalgal productivity was at least as high as in synthetic media, and nutrients uptake efficiency was improved, when replacing different amounts of P and N with struvite. In addition, the possible slow-release of this mineral did not influence the algal growth. Barbera et al. [95] showed also how to efficiently recycle the minerals precipitated after FH, replacing 100% of the P in BG11, both in batch and in continuous cultivation systems, as they obtained growth rates and productivities equal to those of the corresponding controls. It was highlighted that the solubility of MAP is lower than that of other phosphate fertilizers, reporting a maximum of $50\,mg\,L^{-1}$ of P in the cultivation medium, which is anyway largely sufficient to achieve the desired biomass concentration. On the other hand, it was pointed out that, given the N:P molar ratio of MAP (1:1), additional nitrogen needs to be supplied in this case to meet the stoichiometric ratio required by the biomass composition.

7. Bio-products from nutrients

Besides nutrients recycling, it is commonly acknowledged that a comprehensive biorefinery approach is necessary to develop a sustainable and economically feasible process, that exploits the full potential of microalgae biomass for the production of high-value co-products in addition to fuels. Indeed, some conversion pathways discussed in this review can be complemented with the recovery of different valuable products, as summarized in Fig. 2.

For example, by performing a Sequential Hydrothermal Liquefaction (SEQHTL), polysaccharides can be extracted from the biomass, without degradation, through a first step carried out at mild temperatures (e.g. 160 °C), followed by HTL (250–350 °C) for biocrude production (Fig. 3A) [100–102]. In the first extraction step, phosphorus recoveries comparable to conventional HTL are reported, but nitrogen is only 20% of that commonly recovered in AP by the standard one-step process, even though larger amounts could be achieved through the second step [101]. The extracted polysaccharides (α-glucan) are characterized by rheological and thermogravimetric properties that make it suitable for different industrial applications, such as hydrogel formation, fire extinguisher, or also as a promising candidate for thermoplastic starch (TPS), one of the most widely used bio-plastics nowadays [100].

With a slightly different concept, several high-value bio-products can also be obtained from the recovered nutrients. For example, the amino acids and protein-building blocks extracted in the aqueous hydrolyzate from FH can be exploited for industrial applications. Garcia-Moscoso et al. [87] report that arginine, a commercial product used as a food supplement for both human and animal consumption, is found in substantial amounts as a free amino acid in the hydrolyzate. Alternatively, the entire protein-based pool of compounds extracted in the hydrolyzate has been proven as a good feedstock for the production of polyols, by reaction with ethylene diamine and ethylene carbonate. Additionally, it was shown that such polyols could be further used as a raw material for the production of polyurethane foams of characteristics similar to those derived from the petrochemical industry [86]. The production of these proteins-derived valuable compounds could in addition be easily integrated with the MAP precipitation and recycling process proposed by Barbera et al. [95].

Another process currently under development is the precipitation of valuable hydroxyapatite (HAp, i.e. calcium phosphate) by Hydrothermal Mineralization (HTM). For example, Roberts et al. [103] discovered the simultaneous precipitation of HAp nanocrystals and biocrude production under subcritical water treatment of algal biomass.

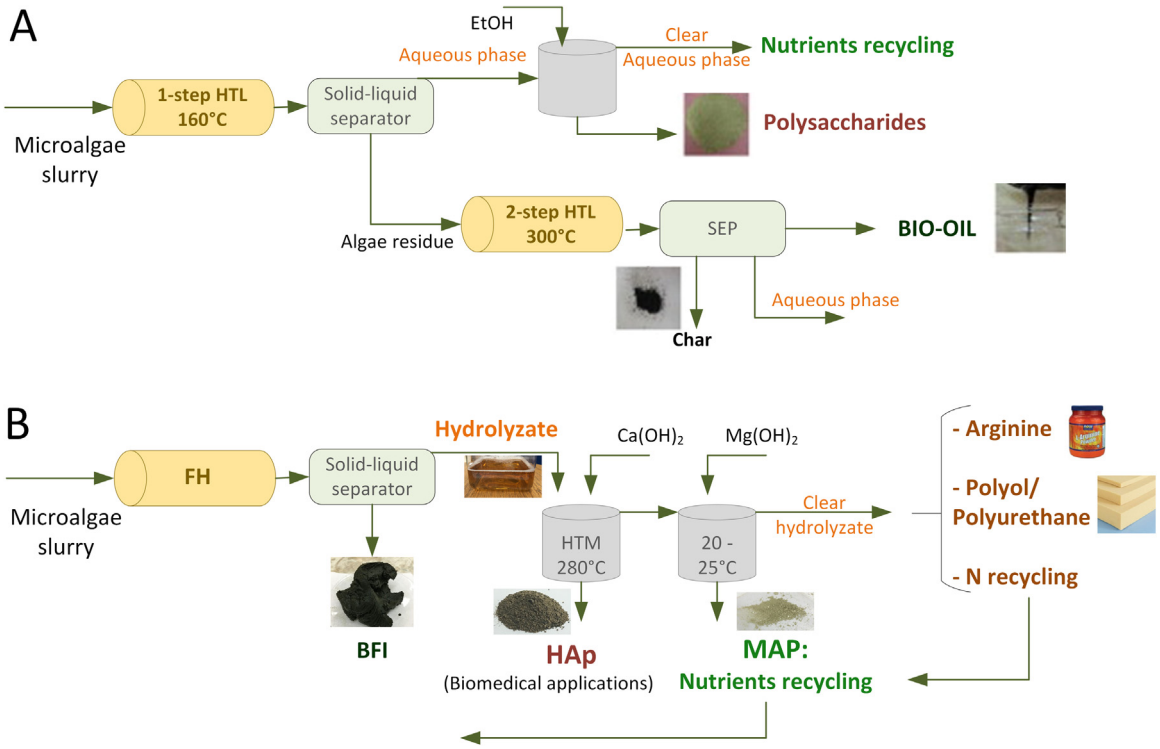

Fig. 3. Integration of nutrients and bio-products recovery in microalgae processing by: A) Sequential-HTL (adapted from [102]) and B) Flash Hydrolysis-Mineralization.

The precipitated HAp was found to promote *in situ* catalytic upgradation of the biocrude, or it could be recovered as a high-value product. The integration of HTM with FH also looks interesting as it only requires the addition of $Ca(OH)_2$ as mineralizer, without any additional heating or pressurization, since the algae hydrolyzate is a hot-compressed liquid directly suitable for HTM [104]. HAp recovered through HTM can find a number of applications, especially in the biomedical sector, such as bone tissue engineering, bioceramic coatings, bone and teeth filler, dental implant coating and others [103,105,106]. The combination of nutrients and valuable co-products recovery following the FH process is schematized in Fig. 3B.

Clearly, when part of the nutrients recovered from the biomass is destined to some high-value bio-product, the extent of recycling to the cultivation system is reduced. However, the market volume of such compounds is generally much lower compared to fuels, so that only a small fraction of recovered nutrients needs to be diverted to these markets, but it is enough to substantially increase the process revenues. Furthermore, by considering the complete Life Cycle Assessment (LCA) of the process, producing such compounds from renewable source positively impacts the overall global fertilizers demands and consumption.

8. Harmful algae blooms

Harmful algal blooms (HABs) occur when colonies of algae grow out of control because of high nutrients levels concentrated in limited areas. HABs have been reported in all 50 states of the United States, with severe impacts on human health and aquatic ecosystems. Nitrogen and phosphorus pollution from human activities makes the problem worse, leading to more frequent and severe blooms, so that algae blooms are becoming a challenging environmental problem. We point out that they could become a resource, as algae slurry from different bloom locations could be collected and processed hydrothermally, producing biofuels intermediates (lipids and carbohydrates) or recovering nutrients/co-products without drying and addition of any chemicals. Recent studies have used HABs as a feedstock mainly for biofuels via a HTL process. For instance, HABs was collected from local ponds and lakes in southeastern Michigan and was used for HTL to extract and quantify biocrude oil yield. The highest biocrude yield (18.5 wt%) was reported from *Cladaphoras* sample collected from Ford Lake [107]. In another investigation, which focuses on the Gulf of Mexico hypoxic area, the net energy balance and economic benefits of harvesting environmental algal blooms and transforming the harvested biomass into biofuels was evaluated. An engineering model was developed to compare the energy efficiency of different harvesting methods and biofuel conversion techniques. Overall, the energetic analysis revealed that the entire harvesting and conversion process can achieve an energy "break-even point" if the chlorophyll concentration is above 55 mg/m^3 [108]. However, these studies have overlooked the advantages of nutrients recovery in the form of valuable minerals, such as struvite or HAp.

9. Material balances and atom economy

Even though the broad experimental work carried out in the latest years proved the feasibility of nutrients recovery and recycling through either AD or HTT, closed-loop nutrients material balances of the whole process are necessary to compare the different conversion routes proposed and to assess their actual viability at large-scale. The results available in the literature are difficult to compare, due to the different calculation basis (e.g. surface area of cultivation system, kg of biomass produced, volume or energy units of fuels production), as well as experimental references and technological choices.

Anyway, some general outcomes can be drawn, highlighting strengths, drawbacks and criticalities of the different pathways. In terms of material balances of the biomass production/conversion process, some nutrients losses occur within the cultivation system, especially for N which can be partially volatilized as free NH_3 (~ 4–5% losses in open ponds are reported) [109,110]. Additionally, roughly 1% of N and P is estimated to be lost as proteins/phospholipids in the extracted oil. In any case, the most important difference among the various processes proposed is the nutrients losses in the downstream units. Rösh et al. report that HTG performed on lipid-extracted algal residues is able to recycle slightly more nutrients than AD [109], especially with

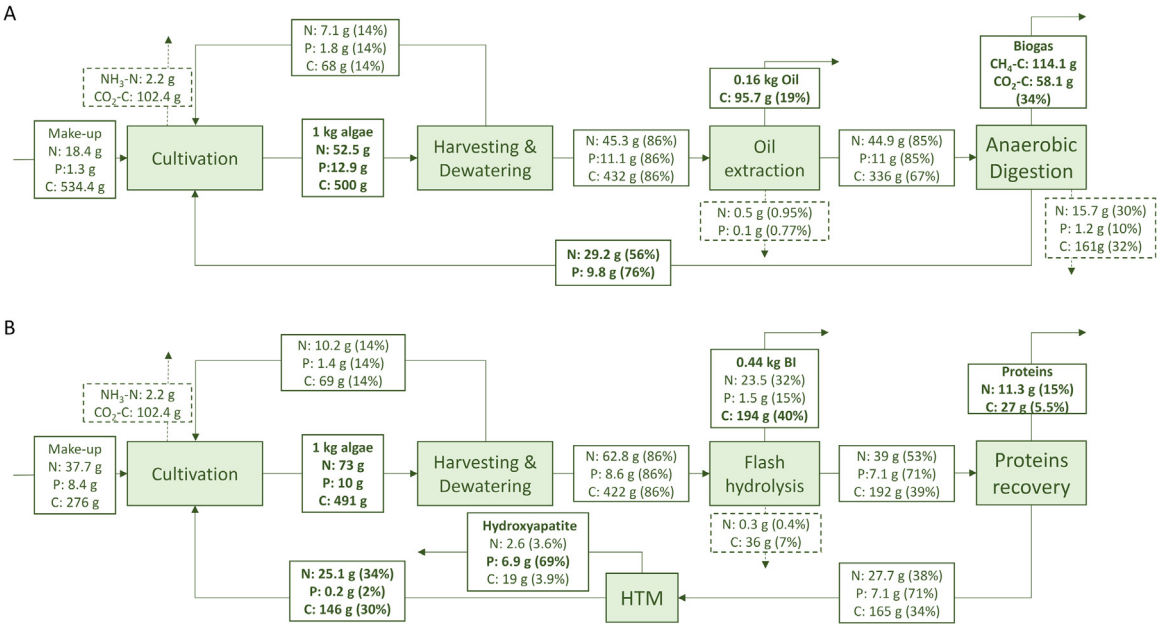

Fig. 4. Material balances and atom economy for the AD [110] (A) and FH [112] (B) pathways. The percentages reported in the brackets refer to the amount contained in 1 kg of algae produced.

respect to N. Besides unconverted biomass, minor losses are related to ammonia volatilization (~ 10%) and P precipitation. This is confirmed also by the analysis of Venteris et al. [23], who claim that lipids extraction followed by catalytic HTG is the most efficient N recovery pathway compared to AD or HTL. On the other hand, HTL appears to perform better for P, as the operating conditions of both AD and HTG lead to insoluble phosphate salts precipitation [23,109,111].

One important point to consider when evaluating different process pathways is the so-called "atom economy", i.e. the yield of specific atoms (in this case N, P and C) into desired products with respect to the corresponding amount present in the starting material. In Fig. 4, two examples are reported, with respect to AD and HTT (FH) conversion pathways respectively.

According to Fig. 4A, AD allows an overall carbon efficiency of 53% as a sum of oil and biogas products, while the remaining fraction is lost in the cultivation step and in the solid residue after the digester. Roughly 30% of the N and 10% of the P are retained in the latter as well [110]. Other works suggest that a higher percentage of P precipitates as insoluble salts [55,56], with overall recoveries in the liquid digestate of 56% and 76% respectively.

As can be seen from the complete material balance shown in Fig. 4B, the N, P and C atom economy following the FH route can be differentiated among different products.

Following the hydrothermal treatment, roughly 40% of the initial C is recovered in the biofuels intermediate product, while 7% is accounted in gaseous products together with some N. The aqueous phase, which can be directly recycled to the cultivation, contains 53% of N, 71% of P and the remaining C (39%), some of which may also be destined to additional high-value products, so that 15% of N and 69% of P could be recovered as proteins or HAp, respectively.

In summary, materials flow analysis indicate that fertilizers consumption can be reduced by 35–40 g N and 4–5 g P per kg of biomass produced through AD [52,110,111], and by 20–40 g N and 5–7 g P through HTL [111]. They are equivalent to 80 and 60 g of urea, and to 20 and 30 g of triple superphosphate, respectively. If onsite nutrients recycling was coupled to the use of wastewaters, N and P fertilizers offsets between 20% and 50% could be achieved [6,23].

10. Concluding remarks

It is now universally acknowledged that the production of microalgal biofuels cannot go without efficient recovery and recycling of nutrients, especially nitrogen and phosphorus. Onsite recovery from biomass and waste streams utilization should be integrated and maximized, with particular attention to the atom economy of the elements of interest, to reduce fertilizers requirements, avoid competition with food production, and overcome other sustainability issues. The intense research efforts carried out in the last decade has laid the foundations for the success of this new approach, showing overall very promising results. However, all the evaluations carried out so far rely on not so many experimental data obtained at laboratory scale, mostly in batch operation mode. Therefore, technological efforts should be directed towards scaling up the processes considered, and optimizing the performances under continuous operation mode, to improve the nutrients recovery and energetic profitability before industrial scale applications can be effectively implemented.

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References

[1] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25:294–306. http://dx.doi.org/10.1016/biotechadv.2007.02.001.
[2] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. Renew Sustain Energy Rev 2010;14:217–32. http://dx.doi.org/10.1016/j.rser.2009.07.020.
[3] Markou G, Vandamme D, Muylaert K. Microalgal and cyanobacterial cultivation: the supply of nutrients. Water Res 2014;65:186–202. http://dx.doi.org/10.1016/j.watres.2014.07.025.
[4] Peccia J, Haznedaroglu B, Gutierrez J, Zimmerman JB. Nitrogen supply is an important driver of sustainable microalgae biofuel production. Trends Biotechnol 2013;31:134–8. http://dx.doi.org/10.1016/j.tibtech.2013.01.010.
[5] Cordell D, Drangert JO, White S. The story of phosphorus: Global food security and food for thought. Glob Environ Chang 2009;19:292–305. http://dx.doi.org/10.1016/j.gloenvcha.2008.10.009.
[6] Canter CE, Blowers P, Handler RM, Shonnard DR. Implications of widespread algal biofuels production on macronutrient fertilizer supplies: nutrient demand and evaluation of potential alternate Nutrient sources. Appl Energy 2015;143:71–80.

http://dx.doi.org/10.1016/j.apenergy.2014.12.065.
[7] Pate R, Klise G, Wu B. Resource demand implications for US algae biofuels production scale-up. Appl Energy 2011;88:3377–88. http://dx.doi.org/10.1016/j.apenergy.2011.04.023.
[8] 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;102:159–65. http://dx.doi.org/10.1016/j.biortech.2010.07.017.
[9] Chisti Y. Constraints to commercialization of algal fuels. J Biotechnol 2013;167:201–14. http://dx.doi.org/10.1016/j.jbiotec.2013.07.020.
[10] Grobbelaar JU. Photosynthetic response and acclimation of microalgae to light fluctuations. In: Algal Cult. Analog. Bloom. Appl. Science Publishers; 2006. p. 671–83.
[11] Gris B, Sforza E, Vecchiato L, Bertucco A. Development of a process for an efficient exploitation of CO2 captured from flue gases as liquid carbonates for Chlorella protothecoides cultivation. Ind Eng Chem Res 2014;53:16678–88. http://dx.doi.org/10.1021/ie502336d.
[12] Eduardo C, Silva DF, Gris B, Sforza E, La N. Effects of sodium bicarbonate on biomass and carbohydrate production in Synechococcus PCC 7002. Appl Biochem Biotechnol 2016;49:241–6. http://dx.doi.org/10.3303/CET1649041.
[13] Gardner RD, Cooksey KE, Mus F, Macur R, Moll K, Eustance E, et al. Use of sodium bicarbonate to stimulate triacylglycerol accumulation in the chlorophyte Scenedesmus sp. and the diatom Phaeodactylum tricornutum. J Appl Phycol 2012;24:1311–20. http://dx.doi.org/10.1007/s10811-011-9782-0.
[14] Uggetti E, Sialve B, Latrille E, Steyer JP. Anaerobic digestate as substrate for microalgae culture: the role of ammonium concentration on the microalgae productivity. Bioresour Technol 2014;152:437–43. http://dx.doi.org/10.1016/j.biortech.2013.11.036.
[15] Gu H, Nagle N, Pienkos PT, Posewitz MC. Nitrogen recycling from fuel-extracted algal biomass: residuals as the sole nitrogen source for culturing Scenedesmus acutus. Bioresour Technol 2015;184:153–60. http://dx.doi.org/10.1016/j.biortech.2014.11.095.
[16] Lowrey J, Armenta RE, Brooks MS. Nutrient and media recycling in heterotrophic microalgae cultures. Appl Microbiol Biotechnol 2016;100:1061–75. http://dx.doi.org/10.1007/s00253-015-7138-4.
[17] Redfield AC. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. Univ. Press Liverpool; 1934. p. 176–92.
[18] Xin L, Hong-ying H, Ke G, Ying-xue S. Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga Scenedesmus sp. Bioresour Technol 2010;101:5494–500. http://dx.doi.org/10.1016/j.biortech.2010.02.016.
[19] Powell N, Shilton A, Chisti Y, Pratt S. Towards a luxury uptake process via microalgae – Defining the polyphosphate dynamics. Water Res 2009;43:4207–13. http://dx.doi.org/10.1016/j.watres.2009.06.011.
[20] Sforza E, Urbani S, Bertucco A. Evaluation of maintenance energy requirements in the cultivation of Scenedesmus obliquus: effect of light intensity and regime. J Appl Phycol 2014;27:1453–62. http://dx.doi.org/10.1007/s10811-014-0460-x.
[21] De Farias Silva CE, Sforza E. Carbohydrate productivity in continuous reactor under nitrogen limitation: effect of light and residence time on nutrient uptake in Chlorella vulgaris. Process Biochem 2016;51:2112–8. http://dx.doi.org/10.1016/j.procbio.2016.09.015.
[22] Shurtz BK, Wood B, Quinn JC. Nutrient resource requirements for large-scale microalgae biofuel production: multi-pathway evaluation. Sustain Energy Technol Assess 2017;19:51–8. http://dx.doi.org/10.1016/j.seta.2016.11.003.
[23] Venteris ER, Skaggs RL, Wigmosta MS, Coleman AM. A national-scale comparison of resource and nutrient demands for algae-based biofuel production by lipid extraction and hydrothermal liquefaction. Biomass Bioenergy 2014;64:276–90. http://dx.doi.org/10.1016/j.biombioe.2014.02.001.
[24] Kim ZH, Park H, Ryu YJ, Shin DW, Hong SJ, Tran HL, et al. Algal biomass and biodiesel production by utilizing the nutrients dissolved in seawater using semi-permeable membrane photobioreactors. J Appl Phycol 2015;27:1763–73. http://dx.doi.org/10.1007/s10811-015-0556-y.
[25] Martínez M. Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus. Bioresour Technol 2000;73:263–72. http://dx.doi.org/10.1016/S0960-8524(99)00121-2.
[26] Umamaheswari J, Shanthakumar S. Efficacy of microalgae for industrial wastewater treatment: a review on operating conditions, treatment efficiency and biomass productivity. Rev Environ Sci Biotechnol 2016;15:265–84. http://dx.doi.org/10.1007/s11157-016-9397-7.
[27] Raposo MFDJ, Oliveira SE, Castro PM, Bandarra NM, Morais RM, Brew JI. On the utilization of microalgae for brewery effluent treatment and possible applications of the produced biomass. J Inst Brew 2010;116:285–92. http://dx.doi.org/10.1002/j.2050-0416.2010.tb00433.x.
[28] Mulbry W, Westhead EK, Pizarro C, Sikora L. Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. Bioresour Technol 2005;96:451–8. http://dx.doi.org/10.1016/j.biortech.2004.05.026.
[29] Park H, Lee C-G. Theoretical calculations on the feasibility of microalgal Biofuels: utilization of marine resources could help realizing the potential of microalgae. Biotechnol J 2016;11:1461–70. http://dx.doi.org/10.1002/biot.201600041.
[30] Hecky RE, Kilham P. Nutrient limitation of phytoplankton in freshwater and marine environments : a review of recent evidence on the effects of enrichment1 Nutritional requirements of phytoplankton Algal cells require elements in relatively. 33. 1988. p. 796–822.
[31] Pohl P, Ohlhase MK, Rautwurst SK, Laus-Kinnerk Baasch K. An inexpensive inorganic medium for the mass cultivation of freshwater microalgae. Phytochemistry 1987;26:1657–9. http://dx.doi.org/10.1016/S0031-9422(00)82264-5.
[32] Ramos Tercero EA, Sforza E, Morandini M, Bertucco A. Cultivation of Chlorella protothecoides with urban wastewater in continuous photobioreactor: biomass productivity and nutrient removal. Appl Biochem Biotechnol 2014;172:1470–85. http://dx.doi.org/10.1007/s12010-013-0629-9.
[33] Ahmad F, Khan AU, Yasar A. The potential of chlorella vulgaris for wastewater treatment and biodiesel production. Pak J Bot 2013;45:461–5.
[34] Singh M, Reynolds DL, Das KC. Microalgal system for treatment of effluent from poultry litter anaerobic digestion. Bioresour Technol 2011;102:10841–8. http://dx.doi.org/10.1016/j.biortech.2011.09.037.
[35] Ramos Tercero EA, Sforza E, Morandini M, Bertucco A. Cultivation of Chlorella protothecoides with urban wastewater in continuous photobioreactor: biomass productivity and nutrient removal. Appl Biochem Biotechnol 2014;172:1470–85. http://dx.doi.org/10.1007/s12010-013-0629-9.
[36] Cai T, Park SY, Racharaks R, Li Y. Cultivation of Nannochloropsis salina using anaerobic digestion effluent as a nutrient source for biofuel production. Appl Energy 2013;108:486–92. http://dx.doi.org/10.1016/j.apenergy.2013.03.056.
[37] Ledda C, Villegas GIR, Adani F, Fernández FGA, Grima EM. Utilization of centrate from wastewater treatment for the outdoor production of Nannochloropsis gaditana biomass at pilot-scale. ALGAL 2015;12:17–25. http://dx.doi.org/10.1016/j.algal.2015.08.002.
[38] Farooq W, Lee Y-C, Ryu B-G, Kim B-H, Kim H-S, Choi Y-E, et al. Two-stage cultivation of two Chlorella sp. strains by simultaneous treatment of brewery wastewater and maximizing lipid productivity. Bioresour Technol 2013;132:230–8. http://dx.doi.org/10.1016/j.biortech.2013.01.034.
[39] Girard J-M, Roy M-L, Hafsa M Ben, Gagnon J, Faucheux N, Heitz M, et al. Mixotrophic cultivation of green microalgae Scenedesmus obliquus on cheese whey permeate for biodiesel production. Algal Res 2014;5:241–8. http://dx.doi.org/10.1016/j.algal.2014.03.002.
[40] Bjornsson WJ, Nicol RW, Dickinson KE, McGinn PJ. Anaerobic digestates are useful nutrient sources for microalgae cultivation: functional coupling of energy and biomass production. J Appl Phycol 2013;25:1523–8. http://dx.doi.org/10.1007/s10811-012-9968-0.
[41] Wang L, Li Y, Chen P, Min M, Chen Y, Zhu J, et al. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. Bioresour Technol 2010;101:2623–8. http://dx.doi.org/10.1016/j.biortech.2009.10.062.
[42] Halim R, Danquah MK, Webley PA. Extraction of oil from microalgae for biodiesel production: a review. Biotechnol Adv 2012;30:709–32. http://dx.doi.org/10.1016/j.biotechadv.2012.01.001.
[43] Ghasemi Naghdi F, González González LM, Chan W, Schenk PM. Progress on lipid extraction from wet algal biomass for biodiesel production. Microb Biotechnol 2016. http://dx.doi.org/10.1111/1751-7915.12360.
[44] Bioenergy Technologies Office (U.S. DoE). Multi-Year Program Plan; 2016. p. 1–108.
[45] Gonzalez-Fernandez C, Sialve B, Molinuevo-Salces B. Anaerobic digestion of microalgal biomass: challenges, opportunities and research needs. Bioresour Technol 2015;198:896–906. http://dx.doi.org/10.1016/j.biortech.2015.09.095.
[46] Ward AJ, Lewis DM, Green FB. Anaerobic digestion of algae biomass: a review. Algal Res 2014;5:204–14. http://dx.doi.org/10.1016/j.algal.2014.02.001.
[47] Bohutskyi P, Ketter B, Chow S, Adams KJ, Betenbaugh MJ, Allnutt FCT, et al. Anaerobic digestion of lipid-extracted Auxenochlorella protothecoides biomass for methane generation and nutrient recovery. Bioresour Technol 2015;183:229–39. http://dx.doi.org/10.1016/j.biortech.2015.02.012.
[48] Bohutskyi P, Chow S, Ketter B, Betenbaugh MJ, Bouwer EJ. Prospects for methane production and nutrient recycling from lipid extracted residues and whole Nannochloropsis salina using anaerobic digestion. Appl Energy 2015;154:718–31. http://dx.doi.org/10.1016/j.apenergy.2015.05.069.
[49] Ramos-Suárez JL, Carreras N. Use of microalgae residues for biogas production. Chem Eng J 2014;242:86–95. http://dx.doi.org/10.1016/j.cej.2013.12.053.
[50] Ayala-Parra P, Liu Y, Field J a, Sierra-Alvarez R. Nutrient recovery and biogas generation from the anaerobic digestion of waste biomass from algal biofuel production. Renew Energy 2017;108:410–6. http://dx.doi.org/10.1016/j.renene.2017.02.085.
[51] Sialve B, Bernet N, Bernard O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol Adv 2009;27:409–16.
[52] Collet P, Hélias Arnaud A, Lardon L, Ras M, Goy RA, Steyer JP. Life-cycle assessment of microalgae culture coupled to biogas production. Bioresour Technol 2011;102:207–14. http://dx.doi.org/10.1016/j.biortech.2010.06.154.
[53] Tercero EAR, Alibardi L, Cossu R, Bertucco A. Anaerobic digestion of microalgal residues to enhance the energetic profit of biocrude production. Chem Eng Trans 2014;37:793–8. http://dx.doi.org/10.3303/CET1437133.
[54] Ehimen EA, Connaughton S, Sun ZF, Carrington GC. Energy recovery from lipid extracted, transesterified and glycerol codigested microalgae biomass. Glob Chang Biol Bioenergy 2009;1:371–81. http://dx.doi.org/10.1111/j.1757-1707.2009.01029.x.
[55] Keymer P, Ruffell I, Pratt S, Lant P. High pressure thermal hydrolysis as pretreatment to increase the methane yield during anaerobic digestion of microalgae. Bioresour Technol 2013;131:128–33. http://dx.doi.org/10.1016/j.biortech.2012.12.125.
[56] Sforza E, Barbera E, Girotto F, Cossu R, Bertucco A. Anaerobic digestion of lipid-extracted microalgae: enhancing nutrient recovery towards a closed loop recycling. Biochem Eng J 2017.
[57] Bohutskyi P, Chow S, Ketter B, Fung Shek C, Yacar D, Tang Y, et al. Phytoremediation of agriculture runoff by filamentous algae poly-culture for biomethane production, and nutrient recovery for secondary cultivation of lipid generating microalgae. Bioresour Technol 2016;222:294–308. http://dx.doi.org/

10.1016/j.biortech.2016.10.013.
[58] Prajapati SK, Kumar P, Malik A, Vijay VK. Bioconversion of algae to methane and subsequent utilization of digestate for algae cultivation: a closed loop bioenergy generation process. Bioresour Technol 2014;158:174–80. http://dx.doi.org/10.1016/j.biortech.2014.02.023.
[59] Alcántara C, García-Encina PA, Muñoz R. Evaluation of mass and energy balances in the integrated microalgae growth-anaerobic digestion process. Chem Eng J 2013;221:238–46. http://dx.doi.org/10.1016/j.cej.2013.01.100.
[60] Patel B, Guo M, Izadpanah A, Shah N, Hellgardt K. A review on hydrothermal pretreatment technologies and environmental profiles of algal biomass processing. Bioresour Technol 2016;199:288–99. http://dx.doi.org/10.1016/j.biortech.2015.09.064.
[61] López Barreiro D, Prins W, Ronsse F, Brilman W. Hydrothermal liquefaction (HTL) of microalgae for biofuel production: state of the art review and future prospects. Biomass Bioenergy 2013;53:113–27. http://dx.doi.org/10.1016/j.biombioe.2012.12.029.
[62] Patel B, Guo M, Izadpanah A, Shah N, Hellgardt K. A review on hydrothermal pretreatment technologies and environmental profiles of algal biomass processing. Bioresour Technol 2016;199:288–99. http://dx.doi.org/10.1016/j.biortech.2015.09.064.
[63] Onwudili JA, Lea-Langton AR, Ross AB, Williams PT. Catalytic hydrothermal gasification of algae for hydrogen production: composition of reaction products and potential for nutrient recycling. Bioresour Technol 2013;127:72–80. http://dx.doi.org/10.1016/j.biortech.2012.10.020.
[64] Patzelt DJ, 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 2014;27:2239–50. http://dx.doi.org/10.1007/s10811-014-0496-y.
[65] Onwudili JA, Lea-Langton AR, Ross AB, Williams PT. Catalytic hydrothermal gasification of algae for hydrogen production: composition of reaction products and potential for nutrient recycling. Bioresour Technol 2013;127:72–80. http://dx.doi.org/10.1016/j.biortech.2012.10.020.
[66] Patzelt DJ, Hindersin S, Elsayed S, Boukis N, Kerner M, Hanelt D. Microalgal growth and fatty acid productivity on recovered nutrients from hydrothermal gasification of Acutodesmus obliquus. Algal Res 2015;10:164–71. http://dx.doi.org/10.1016/j.algal.2015.05.002.
[67] Patzelt DJ, Hindersin S, Elsayed S, Boukis N, Kerner M, Hanelt D. Microalgal growth and fatty acid productivity on recovered nutrients from hydrothermal gasification of Acutodesmus obliquus. Algal Res 2015;10:164–71. http://dx.doi.org/10.1016/j.algal.2015.05.002.
[68] Elliott DC. Review of recent reports on process technology for thermochemical conversion of whole algae to liquid fuels. Algal Res 2016;13:255–63. http://dx.doi.org/10.1016/j.algal.2015.12.002.
[69] Lòpez Barreiro D, Samorì C, Terranella G, Hornung U, Kruse A, Prins W. Assessing microalgae biorefinery routes for the production of biofuels via hydrothermal liquefaction. Bioresour Technol 2014;174:256–65. http://dx.doi.org/10.1016/j.biortech.2014.10.031.
[70] López Barreiro D, Bauer M, Hornung U, Posten C, Kruse A, Prins W. Cultivation of microalgae with recovered nutrients after hydrothermal liquefaction. Algal Res 2015;9:99–106. http://dx.doi.org/10.1016/j.algal.2015.03.007.
[71] Bagnoud-Velásquez M, Schmid-Staiger U, Peng G, Vogel F, Ludwig C. First developments towards closing the nutrient cycle in a biofuel production process. Algal Res 2015;8:76–82. http://dx.doi.org/10.1016/j.algal.2014.12.012.
[72] Heilmann SM, Jader LR, Harned LA, Sadowsky MJ, Schendel FJ, Lefebvre PA, et al. Hydrothermal carbonization of microalgae II. Fatty acid, char, and algal nutrient products. Appl Energy 2011;88:3286–90. http://dx.doi.org/10.1016/j.apenergy.2010.12.041.
[73] Levine RB, Sambolin Sierra CO, Hockstad R, Obeid W, Hatcher PG, Savage PE. The use of hydrothermal carbonization to recycle nutrients in algal biofuel production. Environ Prog Sustain Energy 2013;32:962–75. http://dx.doi.org/10.1002/ep.11812.
[74] Yao C, Pan Y, Lu H, Wu P, Meng Y, Cao X, et al. Utilization of recovered nitrogen from hydrothermal carbonization process by Arthrospira platensis. Bioresour Technol 2016;212:26–34. http://dx.doi.org/10.1016/j.biortech.2016.03.166.
[75] Garcia Alba L, Torri C, Fabbri D, Kersten SRA, (Wim) Brilman DWF. Microalgae growth on the aqueous phase from hydrothermal liquefaction of the same microalgae. Chem Eng J 2013;228:214–23. http://dx.doi.org/10.1016/j.cej.2013.04.097.
[76] Valdez PJ, Nelson MC, Wang HY, Lin XN, Savage PE. Hydrothermal liquefaction of Nannochloropsis sp.: systematic study of process variables and analysis of the product fractions. biomass and bioenergy. 2012;46:317–31. http://dx.doi.org/10.1016/j.biombioe.2012.08.009.
[77] Hognon C, Delrue F, Texier J, Grateau M, Thiery S, Miller H, et al. Comparison of pyrolysis and hydrothermal liquefaction of Chlamydomonas reinhardtii. Growth studies on the recovered hydrothermal aqueous phase. Biomass Bioenergy 2015;73:23–31. http://dx.doi.org/10.1016/j.biombioe.2014.11.025.
[78] Jena U, Vaidyanathan N, Chinnasamy S, Das KC. Evaluation of microalgae cultivation using recovered aqueous co-product from thermochemical liquefaction of algal biomass. Bioresour Technol 2011;102:3380–7. http://dx.doi.org/10.1016/j.biortech.2010.09.111.
[79] Aida TM, Maruta R, Tanabe Y, Oshima M, Nonaka T, Kujiraoka H, et al. Nutrient recycle from defatted microalgae (Aurantiochytrium) with hydrothermal treatment for microalgae cultivation. Bioresour Technol 2017;234:476–7. http://dx.doi.org/10.1016/j.biortech.2017.03.071.
[80] Biller P, Ross AB, Skill SC, Lea-Langton A, Balasundaram B, Hall C, et al. Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process. Algal Res 2012;1:70–6. http://dx.doi.org/10.1016/j.algal.2012.02.002.
[81] Heilmann SM, Davis HT, Jader LR, Lefebvre PA, Sadowsky MJ, Schendel FJ, et al. Hydrothermal carbonization of microalgae. Biomass Bioenergy 2010;34:83475–82. http://dx.doi.org/10.1016/j.biombioe.2010.01.032.
[82] Yao C, Pan Y, Lu H, Wu P, Meng Y, Cao X, et al. Utilization of recovered nitrogen from hydrothermal carbonization process by Arthrospira platensis. Bioresour Technol 2016;212:26–34. http://dx.doi.org/10.1016/j.biortech.2016.03.166.
[83] Yao C, Wu P, Pan Y, Lu H, Chi L, Meng Y, et al. Evaluation of the integrated hydrothermal carbonization-algal cultivation process for enhanced nitrogen utilization in Arthrospira platensis production. Bioresour Technol 2016;216:381–90. http://dx.doi.org/10.1016/j.biortech.2016.05.110.
[84] Teymouri A, Barbera E, Sforza E, Morosinotto T, Bertucco A, Kumar S. Integration of biofuels intermediates production and nutrients recycling in the processing of a marine algae. AIChe J 2016.
[85] Garcia-Moscoso JL, Obeid W, Kumar S, Hatcher PG. Flash hydrolysis of microalgae (Scenedesmus sp.) for protein extraction and production of biofuels intermediates. J Supercrit Fluids 2013;82:183–90. http://dx.doi.org/10.1016/j.supflu.2013.07.012.
[86] Kumar S, Hablot E, Moscoso JLG, Obeid W, Hatcher PG, DuQuette BM, et al. Polyurethanes preparation using proteins obtained from microalgae. J Mater Sci 2014;49:7824–33. http://dx.doi.org/10.1007/s10853-014-8493-8.
[87] Garcia-Moscoso JL, Teymouri A, Kumar S. Kinetics of peptides and arginine production from microalgae (*Scenedesmus sp.*) by flash hydrolysis. Ind Eng Chem Res 2015;54:2048–58. http://dx.doi.org/10.1021/ie5047279.
[88] Yao C, Wu P, Pan Y, Lu H, Chi L, Meng Y, et al. Evaluation of the integrated hydrothermal carbonization-algal cultivation process for enhanced nitrogen utilization in Arthrospira platensis production. Bioresour Technol 2016;216:381–90. http://dx.doi.org/10.1016/j.biortech.2016.05.110.
[89] Teymouri A, Kumar S, Barbera E, Sforza E, Bertucco A, Morosinotto T. Integration of biofuels intermediates production and nutrients recycling in the processing of a marine algae. Am Inst Chem Eng J 2017;63:1494–502. http://dx.doi.org/10.1002/aic.15537.
[90] Erkelens M, Ward AJ, Ball AS, Lewis DM. Microalgae digestate effluent as a growth medium for Tetraselmis sp. in the production of biofuels. Bioresour Technol 2014;167:81–6. http://dx.doi.org/10.1016/j.biortech.2014.05.126.
[91] Garcia Alba L, Vos MP, Torri C, Fabbri D, Kersten SRA, Brilman DWF. Recycling nutrients in algae biorefinery. ChemSusChem 2013;6:1330–3. http://dx.doi.org/10.1002/cssc.201200988.
[92] Du Z, Hu B, Shi A, Ma X, Cheng Y, Chen P, et al. Cultivation of a microalga Chlorella vulgaris using recycled aqueous phase nutrients from hydrothermal carbonization process. Bioresour Technol 2012;126:354–7. http://dx.doi.org/10.1016/j.biortech.2012.09.062.
[93] Talbot C, Garcia-Moscoso J, Drake H, Stuart BJ, Kumar S. Cultivation of microalgae using flash hydrolysis nutrient recycle. Algal Res 2016;18:191–7. http://dx.doi.org/10.1016/j.algal.2016.06.021.
[94] Barbera E, Sforza E, Kumar S, Morosinotto T, Bertucco A. Cultivation of Scenedesmus obliquus in liquid hydrolysate from flash hydrolysis for nutrient recycling. Bioresour Technol 2016;207:59–66. http://dx.doi.org/10.1016/j.biortech.2016.01.103.
[95] Barbera E, Teymouri A, Bertucco A, Stuart BJ, Kumar S. Recycling minerals in microalgae cultivation through a combined flash hydrolysis-precipitation process. ACS Sustain Chem Eng 2017;5:929–35. http://dx.doi.org/10.1021/acssuschemeng.6b02260.
[96] Haiduc AG, Brandenberger M, Suquet S, Vogel F, Bernier-Latmani R, Ludwig C. SunCHem: an integrated process for the hydrothermal production of methane from microalgae and CO_2 mitigation. J Appl Phycol 2009;21:529–41. http://dx.doi.org/10.1007/s10811-009-9403-3.
[97] Elliott DC, Hart TR, Neuenschwander GG, Rotness LJ, Olarte MV, Zacher AH. Chemical processing in high-pressure aqueous environments. 9. Process development for catalytic gasification of algae feedstocks. Ind Eng Chem Res 2012;51:10768–77. http://dx.doi.org/10.1021/ie300933w.
[98] Moed NM, Lee DJ, Chang JS. Struvite as alternative nutrient source for cultivation of microalgae Chlorella vulgaris. J Taiwan Inst Chem Eng 2015;56:73–6. http://dx.doi.org/10.1016/j.jtice.2015.04.027.
[99] Davis RW, Siccardi AJ, Huysman ND, Wyatt NB, Hewson JC, Lane TW. Growth of mono- and mixed cultures of Nannochloropsis salina and Phaeodactylum tricornutum on struvite as a nutrient source. Bioresour Technol 2015;198:577–85. http://dx.doi.org/10.1016/j.biortech.2015.09.070.
[100] Chakraborty M, McDonald AG, Nindo C, Chen S. An α-glucan isolated as a co-product of biofuel by hydrothermal liquefaction of Chlorella sorokiniana biomass. Algal Res 2013;2:230–6. http://dx.doi.org/10.1016/j.algal.2013.04.005.
[101] Martinez-fernandez JS, Chen S. Sequential Hydrothermal Liquefaction characterization and nutrient recovery assessment. Algal Res 2017;25:274–84. http://dx.doi.org/10.1016/j.algal.2017.05.022.
[102] Chakraborty M, Miao C, McDonald A, Chen S. Concomitant extraction of bio-oil and value added polysaccharides from Chlorella sorokiniana using a unique sequential hydrothermal extraction technology. Fuel 2012;95:63–70. http://dx.doi.org/10.1016/j.fuel.2011.10.055.
[103] Roberts GW, Sturm BSM, Hamdeh U, Stanton GE, Rocha A, Kinsella TL, et al. Promoting catalysis and high-value product streams by in situ hydroxyapatite crystallization during hydrothermal liquefaction of microalgae cultivated with reclaimed nutrients. Green Chem 2015;17:2560–9. http://dx.doi.org/10.1039/c5gc00187k.
[104] Teymouri A, Stuart B, Kumar S. Kinetics of phosphate precipitation from the nutrients-rich algal hydrolyzate. In: Present. 253rd Am. Chem. Soc. Natl. Meet; 2017.

[105] Felicio-Fernandes G, Laranjeira MCM. Calcium phosphate biomaterials from marine algae. Hydrothermal synthesis and characterisation. Quim Nova 2000;23:441–6.
[106] Scarano A, Perrotti V, Degidi M, Piattelli A, Iezzi G. Bone regeneration with algae-derived hydroxyapatite: a pilot histologic and histomorphometric study in rabbit tibia defects. Int J Oral Maxillofac Implants 2012;27:336–40.
[107] Goodwyn A. Biofuel yields of various freshwater nuisance algae (presented at Emerging Researchers National (ERN) Conference); 2016.
[108] Kuo C-T, Schideman L. Harvesting natural algal blooms for concurrent biofuel production and hypoxia mitigation: case study of the Gulf of Mexico situation. In: Proc. Water Environ. Fed; 2011. p. 404–17.
[109] Rösch C, Skarka J, Wegerer N. Materials flow modeling of nutrient recycling in biodiesel production from microalgae. Bioresour Technol 2012;107:191–9. http://dx.doi.org/10.1016/j.biortech.2011.12.016.
[110] Yuan J, Kendall A, Zhang Y. Mass balance and life cycle assessment of biodiesel from microalgae incorporated with nutrient recycling options and technology uncertainties. GCB Bioenergy 2015;7:1245–59. http://dx.doi.org/10.1111/gcbb.12229.
[111] Zhang Y, Kendall A, Yuan J. A comparison of on-site nutrient and energy recycling technologies in algal oil production. Resour Conserv Recycl 2014;88:13–20. http://dx.doi.org/10.1016/j.resconrec.2014.04.011.
[112] Teymouri A, Talbot C, Samaratunga A, Stuart BJ, Kumar S. Maximizing atom (N, P, & C) economy via process integration in the conversion of microalgae to bio-products and biofuels. In: Present. 253rd Am. Chem. Soc. Natl. Meet; 2017.