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Review article

Algal cellulose, production and potential use in plastics: Challenges and opportunities

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ABSTRACT

The development of new biodegradable plastic materials from renewable sources is a major challenge for plastic industries to provide sustainable alternatives to petroleum plastic. Bio-based plastics are generally obtained from photosynthetic biomass such as higher plants, crops and more recently algae. This review aims to summarise the current state of bioplastic production in general with particular emphasis on algal cellulose and its derivatives (nanocellulose) for bioplastic applications. Despite the potential of algae as a feedstock for nanocellulose, little information is available on strain selection regarding cellulose content and downstream processing. This study lists possible optimization opportunities to increase the cellulose yield of algal biomass and the current status of its conversion to nanocellulose. Moreover, the findings of this review provide insight into existing knowledge and future direction in the algal cellulose bioplastic domain based on algal bioplastic life cycle assessment studies. Finally, the review gives an overview of the main standards used for biodegradability certifications in view to limit the access to the biodegradable label when the required quality is not reached.

1. Introduction

It is incontestable that plastics play an important role in our modern society. Unfortunately, the social and economic benefits of plastics come with severe environmental detriment [1]. Since its commercial development in the 1950s, fossil-based plastics have caused serious environmental problems leading to the onset of an imbalance on the equilibrium of our ecosystem. In 2018, the plastic industry produced 359 million metric tons (Mt) of plastic, nearly half of which was utilised for single-use and packaging materials. Moreover, only 25% of plastic waste generated was recycled, 22% being disposed to landfills and/or incineration while the remaining 42% is assumed to have been lost into the environment directly or indirectly, e.g., oceans, non-controlled landfills [2]. Due to the disparity and lack of efficient plastic waste management infrastructures, and the so-called “throw away mentality”, 8 million tons (Mt) of plastic waste end up in the ocean annually [3]. In addition, the greenhouse gas (GHG) emissions from the plastic lifecycle contribute up to 15% towards the total global carbon emission [2].

Under the effect of abiotic factors, littered plastics tend to decompose slowly into plastic debris. Subsequently, the formed microplastic particles (sizes from 1 mm to 1 μm) extensively pollute our marine environment at all levels e.g., smothering marine organisms that make up the base of our food chain [4]. If the current strong growth of plastic usage continues as expected, by 2025, the ocean will accumulate 1 ton of

Abbreviations: ABA, abscisic acid; BR, brassinosteroids; CesA, cellulose synthase; CF-PBR, Circular flow photobioreactor; CK, cytokinins; CNC, cellulose nanocrystals; CNF, Cellulose nanofibrils; ETH, ethylene; GA, gibberellins; GHG, greenhouse gas; GOBASE, Organelle Genome Database; IAA, indole acetic acid; IBA, indolebutyric acid; IM, indomethacin; LCA, life cycle assessment; MFC, microfibrillated cellulose; Mt, metric tons; NAA, naphthylacetic acid; NC, nanocellulose; NCBI, National Center for Biotechnology Information; NFC, nanofibrillated cellulose; NGOs, non-governmental organizations; OPR, open pond reactor; PBAT, polybutylene adipate terephthalate; PBR, photobioreactor; PBS, polybutylene succininate; PCL, polycaprolactone; PHAs, poly-hydroxy-alkanones; PLA, poly-lactic acid; PVA, polyvinyl alcohol; SA, salicylic acid; SDGs, sustainable development goals; SL, strigolactones; TAG, triacylglycerol; TGs, terminal complexes; UN, United Nation; WVP, water vapour permeability.

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plastic for every 3 tons of fish, and by 2050, the mass of plastics in
the ocean will be greater than that of fish [5]. These are some serious
environmental repercussions that will without doubt cause irreversible
and unmanageable damage to marine life, and consequently human life
too.

Under pressure from non-governmental organizations (NGOs) and
awareness of consumers, many companies that utilise plastics in their
products are now looking at their plastic policy in terms of recyclability
and pushing towards a new generation of easily recyclable products with
view of “Design to recycle”. However, plastic recycling is not such a
straight-forward process as it requires complex infrastructure and
diverse waste-streams to separate different types of plastics that cannot
be melted together. Without separating the plastic according to their
chemical composition, the resulting material will exhibit structural
weaknesses rendering the recycled product with limited application.
Furthermore, recycling can become counterproductive when biode-
gradable and non-biodegradable plastics are not differentiated during
the separation and recycling process. Plastic polymers are generally
blended with other functional additives such as antistatic agents, dyes,
stabilizers, flame retardants, etc., to improve their performance (flexi-
bility, resistance, etc.). These chemicals create additional recycling
problems as some of them are known to be toxic and not compatible for
all applications. Moreover, some plastic additives are known to exert
endocrine-disruptive activities that cause reproductive disorders in
human and animals and can be responsible for an increased risk of
cancer development [6]. A list of the most commonly used additives in
plastic materials and their functionalities is outlined in Hahladakis et al.
2018 [7].

In order to progress from non-renewable and non-recyclable plastics
to cost-efficient bio-based materials with reduced environmental foot-
prints, it is envisioned that new bioplastic materials should be aligned
with the United Nation’s (UN) sustainable development goals (SDGs)
[8].

By definition, bioplastics are plastics made from organic renewable
sources such as terrestrial crop biomass, agricultural waste and fast-
growing microorganisms. The term bioplastics can be misleading as it
can refer to plastic material that is either biodegradable or bio-based or
both as shown in Fig. 1. It should be noted that biodegradable plastic
may not necessarily be bio-based and vice versa. For instance, poly-
butylene succinate (PBS), polybutylene adipate terephthalate (PBAT)
and polycaprolactone (PCL) are biodegradable plastics obtained from
fossil sources. Although biodegradable fossil-based polymers look
interesting as an alternative, their production from petrochemicals dis-
qualifies them from carrying the label of CO₂ neutral. Other plastics are
referred as oxo-degradable due to the addition of some chemicals that
accelerate their degradation (prooxidant additive) [9]. Nevertheless,
oxo-degradable plastics are far from being a sustainable solution for the
environment. Incorporated additives are generally insufficient to pro-
provide complete mineralization of the plastic, thus the non-decomposed
fraction will contribute to microplastic pollution [10], and still persist
in the environment. Unfortunately, biodegradable bioplastics generally
possess poor mechanical properties such as tensile strength, oxygen and
water permeability which are relevant parameters to determine the
suitability for different applications [11]. In order to meet the minimum

![Fig. 1. Example of bioplastics currently utilised, segmented by feedstock type and biodegradability. Adapted from [18].](image-url)
application requirements without compromising the biodegradability of the bioplastics, renewable additives should partially be accounted within the novel material in order to meet the current expected performance standard of bioplastics.

Bio-based and biodegradable biopolymer such as cellulose, starch, alginate, chitin, polyactic acid (PLA), chitosan, and their derivatives, revealed to be promising candidates with regards to their abundant availability from numerous resources and their biodegradability [12]. Among them, cellulose is by far the most abundant biopolymer obtained from nature and present in almost all photosynthetic organisms such as plants, algae, tunicates, as well as some bacteria. Moreover, technological advancement can now produce advanced cellulose biомaterials by manipulation of native cellulose fibre at the nano scale. In particular, cellulose fibre has outstanding strength properties with high stiffness (an elastic modulus up to 220 GPa) and tensile strength (around 3 GPa), light weight and low density (around 1.6 g cm$^{-3}$) that can be used to improve the mechanical properties of bioplastics without affecting the biodegradability process [13]. Depending on the extraction and conversion procedure applied on the cellulosic biomass, two types of nanocellulose (NC) can be extracted from cellulose: cellulose nanocrystals (CNC), and cellulose nanofibrils (CNF). The latter is also called nanofibrillated cellulose (NFC) or microfibrillated cellulose (MFC) depending on the field of application.

These nanocellulosic materials exhibit interesting mechanical properties, which are different than their cellulosic precursors present in the source biomass material, and thus provides an opportunity to explore their properties as novel biomaterials for potentially wider bioplastic applications. Several studies have shown that NC can enhance the mechanical properties of bioplastic when coated or incorporated with other biopolymers. For instance, when NC was added in the formulation of starch-based edible films bioplastic for food packaging application, the result showed an improvement in water vapour permeability (WVP) and the mechanical strength of the film [14]. Despite the useful features of NC, its production is energy-intensive, consisting of different mechanical and chemical steps to remove lignin and defibrillate cellulose fibres. As a consequence, this high energy consumption remains a barrier towards commercialisation of NCs [15].

Based on the original source of the biopolymer, we can distinguish three generation of bioplastics: 1st generation bioplastics derived from agricultural crop-based feedstocks (corn, soy, sugar cane) and lignocellulosic biomass (trees). Typical biopolymers extracted from 1st generation feedstocks are mainly cellulose, starch, protein, and further bioprocessed polymers such as PLA, poly-hydroxy-alkanoates (PHAs), and NC. Bioplastics of 1st generation require the use of arable land, nutrients and freshwater, thus competing with food production and consequently not fully aligning with the UN SDGs. The switch to bioplastics using these sources has the potential to affect the world’s food supply while sourcing materials from forests will impact the biodiversity and potentially result in more deforestation.

In order to overcome the detrimental properties associated with 1st generation bioplastics, 2nd generation bioplastics incorporated agricultural waste as well as discarded biomass as a feedstock. All of the above listed biopolymers from 1st generation can also be produced from 2nd generation feedstock. However, these resources are limited and insufficient in term of volume and quality to replace global plastic demand with bioplastics [16]. The disruption presented in the supply chain associated with such feedstock also prevents wide scale commercial opportunities.

Lastly, 3rd generation bioplastics produced from fast-growing microorganism such as bacteria, and specifically microalgae can theoretically address the growing problems related to biomass production as they can be located on non-arable land. Microalgae is of particular interest since it has high growth rates compared to terrestrial plants and can use CO$_2$ rich off-gases, saline and/or wastewater, allowing efficient nutrient recycling (e.g., nitrogen and phosphorous) [17].

A wide range of biopolymers can theoretically be provided from algal biomass and this includes cellulose and NC. It is envisioned that cellulose-based bioplastic derived from algal biomass could substantially minimise the impact on the environment and food supply compared to older generation feedstock, particularly ones associated with lignocellulosic material. In some cases, highly pure cellulose can be extracted from algal biomass. This could reduce the cost allocated for the chemical treatments needed for the removal of lignin and hemi-celluloses as in the case of wood-derived cellulose.

It is understood that the route forward for a truly renewable, bio-based bioplastic will come in the form of utilising a 3rd generation feedstock. Ultimately, CNF/NFC/MFC based material production is sufficiently advanced to be produced at the technical level, but the lack of a novel feedstock has led to a bottleneck. Microalgal biomass has clear advantages as highlighted above to fulfil this gap but, there is a severe lack of understanding in the field of utilising it for bioplastic production. Attempts to produce bioplastic from algal nanocellulose (CNC or CNF) are very rare. Only one recent study reported the use of algal CNC (red algae waste from agar production) as reinforcing filler in polyvinyl alcohol (PVA) or chitosan-based bio-composite films [19]. In the case of PVA, the addition of algal CNC (8 wt%) improved the tensile strength and elastic modulus of the composite by a factor of three while only minutely affecting the transparency of the film. Other studies suggested that CNC produced from Posidonia sp. [20,21] or CNF from Nannochloropsis sp. [22] could also have interesting properties to be used as reinforcing filler for fibres-reinforced composites. However, the use of algal CNC or CNF should not be limited solely as reinforcing additives in other polymer matrices. CNC and CNF can be used directly to produce self-standing films. Usually, CNF-based films show similar barrier properties (to oxygen or water vapour) than conventional petro-based plastics (e.g., cellophane), and improved barrier properties (e.g., to oxygen) compared to CNC-based films, thus better suited for applications such as packaging [23]. Algal CNF seems to have a substantial, and unexplored potential for use in the production of high-performance bioplastics. Given the plethora of algal species available, the different cultivation options/conditions, extraction/conversion technologies as well as end of life choices, the task associated with understanding the gaps will be based on further research. As such, the following sections will provide a holistic overview of the important advancements made in the field associated with the aforementioned unit operations, ranging from the fundamentals of cellulose production and purification to the end of life scenarios, as well as provide direction where a positive change is lacking.

2. Cellulose feedstocks

2.1. Cellulose in plants, animals and bacteria

Cellulose is a water-insoluble polymer composed of glucose molecules in which individual glucose units are connected via acetal linkages
between the C1 and C4 carbons of the glucopyranose ring (Fig. 2). It can reach a high degree of polymerization with up to 15,000 glucose units [24]. Cellulose also has a distinct hydrophilic and hydrophobic character due to the presence of both equatorial hydroxyl groups and axial hydrogen atoms that confers its stability via the coplanar orientation of the individual glucopyranose rings [25].

Cellulose chains in plants (terrestrial and algae) and some bacteria tend to self-assemble into microfibrils, which undergo further aggregation into larger assemblies, leading to cellulose fibers characterized by their microfibril dimension and hydrogen bonds. Cellulose is the predominant form of cellulose in algae and some bacteria. Different chemical and thermal treatments allow the conversion of cellulose (I) to the other listed form of polymorphs. For instance, cellulose (I) can be converted to cellulose (II) using different concentration of an alkaline solution (mercerization). In this step, the orientations of microfibrils are completely disrupted and the original parallel chain (hydrogen bonding pattern is O6-H-O2) in cellulose (I) is switched to anti-parallel chain (hydrogen bonding is O6-H-O2) in cellulose (II). The conversion of cellulose (II) to cellulose (III) is generally performed using diamin or NH3 (liquid) treatment and results in two sub-allomorph Cellulose (IIa) and cellulose (IIb). Finally, the last polymorph of cellulose can be obtained via hydrothermal conversion of cellulose (III) in the presence of glycerol [27].

2.2. Cellulose in algal biomass

2.2.1. Cellulose biosynthesis pathway in microalgae

Information on the biosynthesis of cellulose, its diversity and evolution of cellulose-synthesizing enzyme complexes (terminal complexes) in microorganisms is a key factor for the industrialization process. According to Tsekos et al. [28], cellulose synthesis is conducted by membrane-bound cellulose synthase terminal complexes (TCS), containing cellulose synthases (CesA). The arrangement of cellulose microfibrils (size, shape, crystallinity, and intra microfibrillar associations) is directly related to the geometry of the terminal complexes (TCS) as shown in Fig. 4. and by Tsekos et al. [28]. Typical arrangement of TCGs in higher plants follows a hexagonal structure commonly known as rosettes structure. However, a more diverse organization of TCGs can be found within the algae domain, this includes rosette, single and multiple rows arrangement [29,30]. Rosette structures are found in Charophyceae, while linear TCS divides into three categories: (i) single rows include brown algae (Phaeophyceae) and some red algae (Rodophyta), (ii) multiple rows are found in Ulvophyceae, Chlorophyceae, some Rodophyta, and Dinophyta, and (iii) diagonally arranged multiple rows can be found in Xanthophyceae [31].

2.2.2. Cellulose potential in algal biomass

a. Cellulose bearing families

Several studies have focused on the content of specific algal products, such as pigments, vitamins, lipids, carbohydrates, and proteins to produce a wide range of products. However, only few showed interests in algae as a source of cellulose or nanocellulose (see Table 4 for details). In this context, the composition of the cell wall, and the content of cell wall compounds (i.e., its purity related to cellulose), is relevant for the quality of extracted nanocellululosic products. Cellulose is the major load-bearing component of the cell walls of plants and several algal taxa [33]. Many algal taxa were reported to contain cellulose and hemicelluloses [33-36] (e.g., xyloglucans, xylans, mannans, (1→3),(1→4)-β-glucan) in their extracellular covering (Table 1), such as the green algae Chlorophyta (e.g., Trebouxiophyceae, Ulotrichophyceae, Chlorophyceae) and Charophyta (e.g., Charophyceae), or the red algae Rhodophyta. In the phylum Ochrophyta, the Phaeophyceae class (brown algae) were generally reported to contain cellulose and hemicelluloses (e.g., sulfated xylofucoglucon), while a more limited number of species from Xanthophyceae (yellow green algae) and Chrysophyceae class (golden algae) were found to use cellulose as structural polysaccharide for their cell wall. In some Dinophyta (thecate dinoflagellates), a complex extracellular covering called amphiesma can also include an armour of cellulose plates (theca). Other matrix polysaccharides may also be found in the cell wall of green, red or brown algae such as pectins, ulvans, agars, carrageenans or alginites [33-35] and can be relevant co-products for increasing the economic viability of algal cellulose production.

However, despite its potential relevance to find adapted extraction methods for cellulose, little information is available on the cell wall ultrastructure of many species and a high structural diversity can be observed, sometimes within the same taxon [37,38]. It is usually known that brown algae contain a thick cell wall composed mainly of alginates and fucoidans, the cellulose fraction (cellulose microfibrils) is generally smaller but plays a crucial role by associating with alginates for structural purposes [35]. The cell wall of red algae generally contains sulphated galactans (agars, carrageenans) which are not always associated with cellulose. For instance, the cell wall of Chondrus crispus was shown to contain a layer of cellulose microfibrils embedded in a matrix of carrageenans [39], while Gracilaria verrucosa is bi-layered with an inner layer of agar and an outer layer mainly composed of cellulose with a small fraction of xylose and mannose [40]. Among green algae, Chlorella (Trebouxiophyceae) is known to have a main fibrillar layer of polysaccharides (cellulose, hemicellulose and pectin blend) which can be protected in some cases by a highly resistant algaenan outer layer [34,41,42]. The diversity of the cell wall structure between and within Chlorella species was found to be even higher. For instance, Yamada et al. [43] differentiated three main types of cell wall structures from different strains of Chlorella vulgaris. A large range of cell wall structures was also observed within the Chlorophyceae family. For example, the cell wall of Scenedesmus quadricauda was shown to be trilaminar where pectic and cellulosic layer can be clearly differentiated and are protected by an algaenan layer [44,45], while Oedogonium bharatich was shown to be a two-layered cell wall composed of a mix of cellulose, pectins and glycoproteins followed by a cellulose-free layer containing extensin and arabinogalactan proteins [46]. A pectic layer was also found in some Charophyta (e.g., Penium mariaeaceum, Chara corallina) where it linked an inner layer of cellulose microfibrils to an outer “Ca2+-pectin” layer [35]. In contrast, pectins were not found in the cell wall of some Ulophyceae, but rather ulvans serving as matrix polysaccharides [35]. Nannochloropsis (Ochrophyta/Eustigmatophyceae) is also known to have a very special cell wall containing a porous inner layer composed of struts connecting the cell membrane to a porous cellulose-based layer coated by an algaenan outer layer [37].

Based on current knowledge in the field, Nannochloropsis sp. is the only microalgae studied for its cellulose production potential from which cellulose has been extracted [22,47]. However, there is not much information about other species, thus, more research is needed to identify other interesting species for cellulose production. Some of these might have a high potential of cellulose concentration, this could include microalgae from the Trebouxiophyceae class such as Chlorella sp. and Oocystis sp. [36,41,48], Chlorophyceae class such as Scenedesmus sp., Coelastrilla sp., Chlorococcum sp., Selenastrum sp. [41,49,51], Charophyta phylum such as Chaetosphaeridium sp. and Staurastrum sp.,
Cellulose allomorphs

Cellulose I (Parallel chains)

Cellulose II (Anti-parallel chains)

Cellulose III

Cellulose IV

Chemical structure

Cellulose sub-allomorphs

Cellulose Iα

Cellulose II

Cellulose IIIα

Cellulose IVα

Cellulose Iβ

Cellulose II

Cellulose IIIβ

Cellulose IVβ

Source of cellulose I

| Source of cellulose I | Iα/Iβ ratio |
|-----------------------|-------------|
| Algae (Valonia)       | 60/40       |
| Plant (Castanea sativa) | 25/75     |

1. NaOH treatment (mercerization)
2. NH₃ (l) liquid or diamine treatment
3. NH₃ (g) evaporation / NaOH treatment (from Cellulose IIIβ to Cellulose II)
4. NH₃ (g) evaporation / Hydrothermal treatment (from Cellulose IIIα to Cellulose Iβ)
5. Thermal treatment 260°C in glycerol

Fig. 3. Cellulose allomorphs production pathways and their crystal geometry [26].
Fig. 4. Organization and morphology of cellulose synthesizing terminal complexes (TCs) in different organisms, adapted from [32].
Chrysophyceae class such as Dinobryon sp. or Dinophyta phylum [35,36,52]. Evidence of cellulose is not clear for Chlamydomonas sp. (Chlorophyceae) as contradictory statements were reported in the literature [53,54]. More exotic species from Coccolithophyceae class such as Emiliania huxleyi or Gephyrocapsa oceanica were known to contain cellulose [36].

Among macroalgae, most of the studies for cellulose production focused on Ulvophyceae class with species such as Ulva sp. (Ulveales order), or Cladophora sp., Valonia sp., Boerjesenia sp. (Cladophorales order) (e.g., [55–58]). Many other species from the Cladophorales order are known to contain a well-organized and highly crystalline cellulose such as Siphonoclada sp., Apochnia sp., Dictyosphaeria sp., Chaetomorpha sp., Rhiizonclonium sp. [36], and might be worth of more extensive studies to identify potential for cellulose production. Several other researchers found good potential in Posidonia sp. (Tracheophyta phylum, Monocots class) from which cellulose has been extracted [20,21]. Nitella sp. (Charophyceae class) was studied mainly for its cell wall properties [36], excluding aspects regarding cellulose extraction. Cellulose seems to be present in most red algae (Rhodophyta phylum) and all brown algae (Phaeophyceae class), with interesting potential re-focused on Ulvophyceae class with species such as Fucus sp., Ulva lactuca, Ulva prolifera, or brown algae Fucus sp., Laminaria sp. A limited amount of species from Xanthophyceae class, such as Tribonema sp. (filamentous microalgae) or Vaucheria sp. (macroalgae) were known to contain cellulose [36].

b. Cellulose, hemicellulose and lignin content in algal biomass

Table 1
Overview of the major polymer found in the extracellular covering of different algal taxa, adapted from [33–35].

| Algal taxa (green algae) | Crystalline polysaccharides | Hemicelluloses | Matrix polysaccharides*
|---------------------------------|-----------------|----------------|-----------------
| Chlorophyta (green algae) | Cellulose | Xyloglucans, xylans, mannans, glucuronan, (1 → 3)-β-glucan, (1 → 3),(1 → 4)-β-glucan | Ulvans, pectins
| Charophyceae (green algae) | Cellulose | Xyloglucans, xylans, mannans, (1 → 3)-β-glucan, (1 → 3),(1 → 4)-β-glucan | Pectins
| Rhodophyta (red algae) | Cellulose, (1 → 4)-β-mannan, (1 → 4)-β-xylan, (1 → 3)-β-xylan | Xylans, mannans, glucosmannans, sultated (1 → 3),(1 → 4)-β-glucan, (1 → 3),(1 → 4)-β-xylan | Agars, carrageenans, porphyran
| Phaeophyceae (brown algae) | Cellulose | Sulfated xyluocogluacan, sulfated xylulocogluconaran, (1 → 3)-β-glucan | Alginites, fucoids
| Dinophyta | Cellulose | – | –

* Matrix polysaccharides may form composite layers including cellulose and/or hemicelluloses, or separate layers. The precise ultrastructure can be species dependant and should be looked on a case by case basis.

Chlorella vulgaris was found to reach 25 wt% cellulose [47], while Chlorella vulgaris ranged between 10 and 47.5 wt% [48]. This notable difference in content could be explained by the large influence of the culture conditions on the constitutive elements of the algae such as cellulose, and highlights the importance of good culturing design needed for maximizing cellulose production. For instance, it was shown in the study of Aguirre and Bassi [48] that the nitrate concentration played an important role on the cellulose content of Chlorella vulgaris (ranging from 10 to 47.5 wt%). Another factor that could potentially nuance the lower cellulose content found in total dry weight of the algae, Nannochloropsis gaditana was found to reach 25 wt% cellulose [47], while Chlorella pyrenoidosa contained poor cellulose amount in its covering (15.4 wt%) [53]. On the other hand, when looking at the total dry weight of the algae, Nannochloropsis gaditana was found to reach 25 wt% cellulose [47], while Chlorella vulgaris ranged between 10 and 47.5 wt% [48]. This notable difference in content could be explained by the large influence of the culture conditions on the constitutive elements of the algae such as cellulose, and highlights the importance of good culturing design needed for maximizing cellulose production. For instance, it was shown in the study of Aguirre and Bassi [48] that the nitrate concentration played an important role on the cellulose content of Chlorella vulgaris (ranging from 10 to 47.5 wt%). Another factor that could potentially nuance the lower cellulose content found in total dry weight of the algae, Nannochloropsis gaditana was found to reach 25 wt% cellulose [47], while Chlorella pyrenoidosa contained poor cellulose amount in its covering (15.4 wt%) [53]. On the other hand, when looking at the total dry weight of the algae, Nannochloropsis gaditana was found to reach 25 wt% cellulose [47], while Chlorella vulgaris ranged between 10 and 47.5 wt% [48]. This notable difference in content could be explained by the large influence of the culture conditions on the constitutive elements of the algae such as cellulose, and highlights the importance of good culturing design needed for maximizing cellulose production. For instance, it was shown in the study of Aguirre and Bassi [48] that the nitrate concentration played an important role on the cellulose content of Chlorella vulgaris (ranging from 10 to 47.5 wt%). Another factor that could potentially nuance the lower cellulose content found in total dry weight of the algae, Nannochloropsis gaditana was found to reach 25 wt% cellulose [47], while Chlorella pyrenoidosa contained poor cellulose amount in its covering (15.4 wt%) [53]. On the other hand, when looking at the total dry weight of the algae, Nannochloropsis gaditana was found to reach 25 wt% cellulose [47], while Chlorella pyrenoidosa contained poor cellulose amount in its covering (15.4 wt%) [53].

Table 2
Cellulose, hemicellulose and lignin content in total dry weight basis of algal feedstock. n.d.: not determined, det.: detected (either directly or indirectly).

| Phylum/class | Strain | Cellulose [%] | Hemicellulose [%] | Lignin [%] | References |
|---------------|--------|---------------|-----------------|------------|------------|
| Microalgae – | Mix of microalgae & cyanobacteria from waste water treatment plant | 7.1 | 16.3 | 1.5 | [61] |
| Chlorophyta/Trebouxioyceae | Chlorella vulgaris | 10–47.5 | n.d. | n.d. | [48] |
| Ochrophyta/Eustigmatophyceae | Nannochloropsis gaditana | 25 | det. | n.d. | [47] |
| Macroalgae Chlorophyta/Ulvophyceae | Cladophora glomerata | 21.6 | det. | n.d. | [55] |
| Chlorophyta/Ulvophyceae | Ulva lactuca | 12.4 | n.d. | n.d. | [56] |
| Chlorophyta/Ulvophyceae | Ulva prolifera | 6.0 | 12.2 | 9.8 | [66] |
| Chlorophyta/Ulvophyceae | Ulva pertusa | 16.6 | 32.5 | 1.5 | [67] |
| Chlorophyta/Ulvophyceae | Fucus vesiculosus, Laminaria digitata | 19.4 | 14.4 | 9.4 | [68] |
| Chlorophyta/Ulvophyceae | Gelidium elegans | 6.7 | 16.8 | n.d. | [69] |
| Ochrophyta/Phaeophyceae | Cystosphaera jacquinotii | 40.7 | 7.1 | 7.9 | [70] |
| Ochrophyta/Phaeophyceae | Fucus vesiculosus, Laminaria digitata | 4.6 | 6.1 | 19 | [71] |
| Rodophyta/Florideophyceae | Posidonia oceanica | 17.2 | 29.5 | 4.5 | [73] |
| Tracheophyta/Monocots | Gelidium elegans | 32.5 | 23.3 | 28.2 | [28] |
| Tracheophyta/Monocots | Fucus vesiculosus, Laminaria digitata | 31.4–40.0 | 21.8–25.7 | 29.3–29.8 | [72] |
| Tracheophyta/Monocots | Posidonia australis | 20.0 | 20.8 | 29.8 | [74] |
were retained or not during the harvesting process. Nanocellulose production might also be considered in a biorefinery approach as proposed by Lee et al. [22], where the cellulose content of *Nannochloropsis oceanica* leftover (after lipid and protein extraction) was 34 wt% and represented 47 wt% of the total carbohydrate content.

Regarding cellulose purity, it was found that *Chlorella pyrenoidosa* can contain two-fold hemicellulose mass, compared to cellulose in its cell wall [53] (Table 3). Again, this does not necessarily mean that *Chlorella* sp. has a poor cellulose purity, as also suggested by the high cellulose content from the study of Aguirre and Bassi [48]. But this is most likely due to poorly optimized culture conditions. Gunnison and Alexander [52] also showed that some microalgae species such as *Staurastrum* sp. (Charophyta/Zygnematophyceae) can contain lignin in the range of 1.2–5.6 wt% of its cell wall, high cellulose content (70 wt% of cell wall mass) and a low hemicellulose fraction (4 wt%). However, no other studies known so far reported any quantification of the hemicellulose or lignin content in microalgae species. To build on this, Verveeris et al. [61] provided further insights into this question as they compared the composition of several organic materials, including a freshwater microalgae slime (*Chlorella* sp., *Scenedesmus* sp., and other microalgae and cyanobacteria) collected from a waste water treatment plant, for use as paper pulp supplement. It was found that the slime contained 7.1 wt% cellulose, 16.3 wt% hemicellulose and a low fraction of lignin (1.5 wt%).

It was suggested that the cellulose and hemicellulose content could come from *Scenedesmus* sp., *Ulothrix* sp., *Stigeoclonium* sp. or *Chlorella* sp. It was noted that *Scenedesmus* sp. usually dominates in spring, while the others are more prevalent during autumn and winter [62,63], thus there is some affect from seasonality in such studies.

Compared to microalgae, a larger number of studies is available for the compositional analysis of macroalgae, especially for cellulose, hemicellulose and lignin. Most of the studies assessed macroalgae from the Ulvophyceae class such as *Ulva* sp., *Cladophora* sp., *Valonia* sp. and Phaeophyceae class such as *Fucus* sp. and *Laminaria* sp. (Tables 2 and 3). Some red algae (Rhodophyta) and Tracheophyta such as *Possidonia* sp. were also quantified. The cellulose content is variable depending on the strain, ranging from 4 to 40 wt% of the total dry weight and up to 75 wt% of the dried cell wall for *Valonia* sp. It appears that certain macroalgae such as *Possidonia oceanica* has a significant amount of lignin (around 30 wt%) coupled with a high cellulose content (30–40 wt%). *Ulva* sp. has the advantage of containing less lignin (up to 10 wt%) with a variable but important potential for cellulose (6–40 wt%). Other strains such as *Cladophora* sp. seem to have a great potential for cellulose (20–45 wt%), which is highly crystalline [64]. However, there was no clear evidence of the purity of the cell wall, and contradictory statements regarding the possible existence of lignin was also deduced. Finally, strains such as *Valonia* sp. seem to have a very pure and highly crystalline cellulosic cell wall [64,65]. This suggests that different methods should be used to purify cellulose from other compounds depending on the composition of the algae, and will be developed in a later part of this review.

### Table 3

| Phylum/class | Strain | Cellulose [%] | Hemicellulose [%] | Lignin [%] | References |
|-------------|--------|---------------|------------------|-----------|------------|
| Microalgae  | *Chlorella pyrenoidosa* | 15.4 | 31 | n.d. | [53] |
| Ochrophyta/Eustigmatophyceae | *Nannochloropsis gaditana* | 75 | det. | n.d. | [37] |
| Charophyta/Zygnematophyceae | *Staurastrum* sp. | 72 | 4.0 | 1.2–5.6 | [52] |
| Macroalgae  | *Valonia ventricosa* | 75 | det. | abs. | [65] |
| *Cladophora rupestris* | 28.5 | n.d. | n.d. | [76] |
| *Ulva lactuca* | 19 | det. | n.d. | [76] |
| *Chaetomorpha melagonium* | 41 | det. | n.d. | [76] |
| *Enteromorpha sp.* | 21 | det. | n.d. | [76] |
| *Fucus serratus* | 13.5 | det. | n.d. | [76] |
| *Laminaria digitata* | 20 | det. | n.d. | [76] |
| *Laminaria saccharina* | 18 | det. | n.d. | [76] |
| *Halidrys siliquosa* | 14 | det. | n.d. | [76] |
| *Himanthalia lorea* | 8 | det. | n.d. | [76] |
| *Pilota plumesa* | 24 | det. | n.d. | [76] |
| *Rhodymenia palmata* | 7 | det. | n.d. | [76] |

### Fig. 5

Optimization strategies of algal biomass for nanocellulose production.
3. Optimization of algal biomass for cellulose and nanocellulose production

Herein, we outline different strategies that could potentially increase the overall yield of cellulose produced by microalgae (Fig. 5). One approach for achieving this goal is the optimization of cell culture conditions to improve the growth rate and cell density [77–80]. Obtaining higher biomass yields in shorter times is essential for the use of microalgae at a commercial production scale. Other strategies aim at potentially influencing cellulose synthesis by applying specific stress conditions like low nitrogen, sulphur, or phosphorus, or high salinity [81–83]. It is also possible to influence metabolic pathways directly on a genetic level to drive photosynthesis and CO₂ fixation to higher growth rates and biomass yields, or accumulation of specific products. Newly developing genetic engineering approaches and genome editing technologies are currently paving the way for the construction of industrial microalgal strains with desired and optimized properties [84–90]. Finally, the use of phytohormones either exogenously added or genetically engineered for endogenous expression show great potential of boosting high biomass production, and accumulation of desired products, especially under abiotic stress conditions [91,92].

3.1. Optimization of culture conditions (phototrophic, heterotrophic, mixotrophic)

Achieving high productivity represents a major challenge in commercial applications for microalgae, and consequently also for the production of cellulose at an industrial scale. Microalgae often exhibit remarkable individual flexibility in their mode of cultivation. Whether phototrophically, mixotrophically or heterotrophically grown, each mode represents technical challenges of its own. Mixotrophic culture is gaining increasing attention because the metabolic capability of some microalgae to use both photosynthesis and heterotrophic processes alone [77,79]. Mixotrophic cultivation modes, which combine wastewater treatment with the production of high-value biomass, have future potential to allow large-scale manufacturing of raw materials from microalgae in a cost-effective, and sustainable approach.

In general, there are two possible pathways of carbon fixation in microalgae: (i) autotrophic, which relies on photosynthetic growth and fixation of inorganic carbon (i.e., carbon dioxide) through the Calvin-Benson cycle, and (ii) heterotrophic, based on the assimilation of organic carbon in the absence of light [93]. Some microalgae species show metabolic versatility but more species are obligate photomixotrophs rather than heterotrophs [78]. Heterotrophic culturing of microalgae has been reported to enhance the biomass concentration by as much as 25-fold compared to phototrophic conditions and ultra-high biomass yields of more than 200 g L⁻¹ of culture have been recently reported [79,80]. Nevertheless, a number of commercially relevant microalgae (e.g., Chlorella vulgaris, Arthrospira platensis (former: Spirulina platensis), Chlamydomonas reinhardtii, and Dunaliella salina), were shown to exhibit a more flexible growth mode. For instance, they can also grow heterotrophically and even showed improved biomass yields under mixotrophic conditions as compared to a photomixotrophic or heterotrophic growth mode alone [94–97]. Mixotrophic cultivation of many microalgae strains show additive or synergistic effects of photomixotrophic and heterotrophic metabolic activities, leading to an overall increases in productivity [98–100]. Several studies, which analysed the microalgal energy metabolism, showed that mixotrophic cultures resulted in higher energetic efficiency because the amount of energy dissipated was minimal which presumably increases their productivity [101,102].

The most prominent advantage of mixotrophic or heterotrophic growth, compared to phototrophic cultivation is the possibility to grow microalgae in wastewater to utilise organic carbon, thereby offering an excess of carbon flow in addition to atmospheric inorganic CO₂ for cost-effective biomass production and extracellular polysaccharide accumulation [103,104]. To improve the nutrient uptake of microalgae in mixotrophic cultivation, low-strength ultrasound has been used to increase the transport of nutrients into algal cells that consequently promoted biomass yields [105]. Ideally, the carbon present in wastewater should be utilised by algae, negating the need of an exogenous source. Typically, municipal waste water contains <10% sugars, with the majority of carbon sources linked to protein or/and fibre [106]. In order to liberate C for microalgae uptake, a co-culture of bacteria is needed to ensure release what is the correct growth phase [107], presumably under heterotrophic conditions. It should be noted that not all microalgae species are suitable for use in wastewater treatment and a general consensus is not available. Studies conducted thus far have not been conclusive for direct comparison due to variability in experimental conditions [107] and as such the cultivation of different microalgae species in wastewater should not be treated equally without taking the environmental factors into account.

3.2. Specific stress conditions

Eukaryotic microalgae possess remarkable adaptability to most abiotic stresses. Evidence for stress-induced increase in the cellulose content of microalgae is rare [81] and may deserve more attention. Here, we focus on specific stress conditions, which can be purposely applied to stimulate the overall carbohydrate production, and could potentially be linked to increased cellulose synthesis. Future optimization of algal biomass for cellulose production would require specifically targeting the cellulose compound for quantification. Common stress strategies for carbohydrate increase include nutrient limitation/depletion (e.g., nitrogen, phosphorus, sulphur), or high salinity (osmotic stress) [82,83]. A decrease of temperature below optimal conditions was also reported to improve the carbohydrate content of Chlorella vulgaris [108], or excessive light intensity (photo inhibition) slightly increased the glucose content of Scenedesmus obliquus [109] at the cost of decreased growth rates. However, the efficiency and energy consumption of such techniques would make them difficult to implement for large-scale carbohydrate production.

3.2.1. Medium induced stress by deprivation or excess of specific compounds

One of the main interest for microalgae cultivation under nutrient limitation/depletion was the promotion of lipids and other added value products, including carbohydrates [110–112]. Microalgae grown under nitrogen limitation generally tend to redirect their carbon flow from protein synthesis to lipid or carbohydrate synthesis pathways [113]. This effect was observed for Nannochloropsis oceanica, where the proportion of triacylglycerol (TAG) and glucose increased in response to N-depletion [114]. Several studies admitted that glucose accumulated in Nannochloropsis sp. mainly in the form of β-1,3-glucan [114,115], thus targeting specific genes involved in their biosynthesis (e.g., UDPG pyrophosphorylase and β-1,3-glucan synthase). Very recently, Jeong et al. [81] targeted the cell wall evolution and genes involved in cellulose synthesis of Nannochloropsis salina under nitrogen starvation, showing that glucose could also accumulate in the cellulose wall of the cell. The microalgae exhibited substantial thickening of its cell wall cellulosic layer and increased cellulose content when grown under nitrogen deficiency for 2 days (compared conditions: 427.5 mg L⁻¹ NaNO₃ without nitrogen 0 mg L⁻¹ NaNO₃). This effect was reasonably explained by an enhanced transcript level of genes involved in cellulose biosynthesis (e.g., UDPG pyrophosphorylase and cellulose synthases), which was detectable during nitrogen starvation conditions. Nitrogen limitation induced a 1.5-fold increase of the cellulose content compared to non-stressed conditions, respectively, 9% and 6% of the total biomass dry weight, while increasing cell size (1.18-fold increase, from 2.73 μm to 3.24 μm) and cell wall thickness (1.54-fold increase, from 52 nm to 80 nm). Calcofluor white, a common dye for cellulose detection,
revealed that at least some of the cell wall thickening was attributed to an increase of the cellulosic layer. On the other hand, the specific growth rate decreased significantly under stressed conditions (control: 0.53 d\(^{-1}\), nitrogen deficiency: 0.09 d\(^{-1}\)).

Similarly, it would be worthwhile to try other common nutrient starvation strategies used for carbohydrate increase such as low phosphorus or sulphur \cite{82,83} to investigate the extent to which they could promote cellulose synthesis or cytoplasmic sugar accumulation in Nanochloropsis sp. The effect of nitrogen, phosphorus or sulphur depletion on potential cellulose accumulation in other microalgal species still remains unexplored. A clearer picture of the glucose accumulation pathways either in the form of starch or cellulose seems necessary.

A number of studies reported the overall increase of carbohydrates under salinity stress (high salinity) in freshwater species such as Chlorococcum sp. or Scenedesmus sp. \cite{116-118}. Several reviews pointed that microalgae may produce low molecular weight organic compounds (osmolytes). For instance, carbohydrates, to reduce the osmotic stress generated by an increase in salinity \cite{82,113,119}. However, adapting to such changes of turgor pressure could also involve other mechanisms such as variation in cell size or increase in cell wall thickness, respectively, decreasing the tension applied to the cell wall or increasing its tensile strength \cite{120}. Cellulose is known to be the major contributor to cell wall strength in land plants \cite{121}, similarly cellulose bearing microalgae could increase their cellulosic wall thickness to increase the mechanical strength of their cell wall for osmotic acclimation. This topic may deserve more attention in the vision of optimizing cellulose production in microalgae.

### 3.2.2. Growth limitation under stress and possible strategies of mitigation

Stress-induced stimulation of specific compounds is often associated with decreased growth rates caused by oxidative stress \cite{110}. This limitation was addressed by many reviews \cite{82,83,110-112,122}. A trade-off is generally found in terms of stress intensity to maximize the overall productivity of the targeted product. Ideal stress intensities are species dependent. For instance, salinity values ranging from 0.06 mol\(\cdot\)L\(^{-1}\) to 0.4 mol\(\cdot\)L\(^{-1}\) of NaCl increased the carbohydrate fraction of freshwater species such as Scenedesmus obliquus \cite{from 28 wt% to 52 wt% of the total dry weight or Chlorella vulgaris \cite{26 wt% to 47.5 wt% of dry weight} \cite{116}. However, the maximum carbohydrate productivity was reached at 0.3 mol\(\cdot\)L\(^{-1}\) for Scenedesmus \cite{22.2 mg L\(^{-1}\)d\(^{-1}\) and 0.08 mol L\(^{-1}\) for Chlorella \cite{12.6 mg L\(^{-1}\)d\(^{-1}\) due to a differing decline in growth rates.

Different cultivation strategies have been proposed to mitigate the conflict between biomass growth and content of targeted compounds \cite{112,119,122}. These strategies usually involve multi-stage cultivation where ideal growth conditions maximize biomass production, while stress conditions increase the content of specific products. One reported strategy used intermittent nitrogen supply (3 to 10 cycles of starvation) during fed-batch culture \cite{123,124}. The most widely used strategy consists of two-stage cultures where high cell densities are produced prior to application of the stress \cite{112,119,122}. Mixotrophic or heterotrophic cultivation could be envisioned to increase biomass productivity during the first stage \cite{112}. The application of a salinity-gradient, in case of stress from high salinity, was also shown to reduce growth inhibition during the second stage by improving the adaptation of cells to the stress conditions \cite{125}. Conversely, two-stage strategies require higher investment and operating costs since more photobioreactors/ponds and a transfer of the biomass between them would be needed \cite{119}. Additionally, a longer cultivation time is needed in case that two stages is envisioned in the same culturing container, which also avoids the possibility of continuous or semi-continuous cultures. Comparative assessments (e.g., techno-economic) would be required between one- and two-stage culturing strategies to evaluate the most cost-effective solution for cellulose production. Culturing design involving intermediate harvesting (e.g., nutrient depletion) should be avoided to decrease costs \cite{119}. In that sense, strategies such as high salinity could be more cost-effective. Furthermore, stress conditions often have pleiotropic effects in microalgae by influencing not only the carbohydrate production but also lipid, pigments or antioxidant contents and should be envisioned in a biorefinery concept to increase overall economic viability.

### 3.3. Genetic engineering for improving microalgal biomass and productivity

Despite their potential to boost the production of bioproducts, genetic engineering techniques in microalgae have been lagging behind those available for higher plants, fungi and bacteria \cite{84}. However, recent advances in genome sequencing \cite{126} and genome editing technologies \cite{85} promise enhanced biotechnological exploitation of microalgae by genetic engineering of strains with optimized physiological properties (metabolic engineering), making them more suitable for commercial utilisation \cite{84}.

The availability of genomic sequence data is critically required for genome editing approaches. First microalgal nuclear genome sequences were reported from Thalassiosira pseudonana \cite{127}, Chlamydomonas \cite{128} and Phaeodactylum \cite{129}. The number of publicly accessible genomes is currently estimated to range between 40 and 60 and is predicted to significantly increase in the near future \cite{84,130}. Genetic engineering of microalgae may not only involve manipulations of nuclear DNA but also modifications on the level of chloroplast and mitochondrial genomes for which quite a number of sequences are already available at the NCBI organelle database and the organelle genome database GOBASE \cite{131}. In addition to the detailed genome sequence information and the determination of gene functions, also molecular techniques and synthetic toolbox DNA elements as for instance gene delivery methods, plasmids, selectable markers, promoters, ribosome binding sites, transcriptional regulators (enhancer/silencer) and transcription terminators are prerequisites for the genetic engineering of economically viable microalgal strains with desired properties \cite{84,132,133}.

Metabolic pathways can be modified by overexpression or silencing of certain genes to achieve a higher yield of desired products \cite{86}. In this context, significant effort has been undertaken to increase lipid contents and change the fatty acid composition for biofuel production \cite{87}. However, the enhancement of photosynthesis and biomass productivity of microalgae has also been addressed by genome editing. These factors have primarily shown to be determined by light utilisation efficiency and CO\(_2\) fixation rate \cite{88}. First encouraging results on the engineering of microalgal strains for higher photosynthetic efficiency were obtained using the CRISPR-Cas9 method \cite{89}. In another study, the CO\(_2\) fixation capacity of Chlorella vulgaris could be enhanced by a genetic modification, to achieve an enhanced expression of the Calvin cycle enzyme aldolase \cite{90}.

The emergence of genomic sequence databases and the determination of gene-specific functions represent an indispensable basis to allow genetic engineering of microalgae towards increased metabolic output. Moreover, significant recent advances provide molecular tools and synthetic toolbox DNA elements in combination with genome editing techniques, which now allow directed gene manipulation with high precision in shorter duration. All these developments have the potential to shift algal biotechnology towards profitable bioengineered production strains, which can economically compete with other existing systems for the production of bioproducts.

Genetically engineered microalgae using classical recombinant DNA techniques or gene-editing procedures currently remain in the EU under the GMO (Genetically modified organism) legislation. This means that its products must receive approval prior to any commercialisation \cite{134}. Risk assessments related to genetically engineered microalgal strains aim to predict their potential damage on the environment as well as on human and animal health \cite{135}. This includes also the analysis of the risk of their deliberate escape into the environment, which may potentially cause the spreading of recombinant DNA by horizontal gene
transfer to other organisms or to their wild-type counterparts by sexual reproduction. The use and design of transgenes should therefore be considered not to confer a selective advantage in nature. Horizontal gene transfer mediated by viruses for instance is regarded as a rare event but has nevertheless been documented in algae [135,136]. To improve biosafety issues related to genetically modified microalgae, legislative rules should be harmonized, because they are unfortunately not equal around the world [137]. Furthermore, analytical procedures should be adapted to better trace the potential transfer of transgenes and their impact on the natural environment [137].

3.4. Phytohormones for improving biomass yield and stress resistance

Phytohormones comprise diverse classes of low molecular weight chemical messengers in plants that play essential roles as regulators of growth and development. Nine categories of phytohormones have been identified to date comprising: auxins, cytokinins (CK), gibberellins (GA), abscisic acid (ABA), ethylene (ETH), brassinosteroids (BR), salicylates (SA), jasmonates (JA) and strigolactones (SL) [138]. These natural compounds occur and act at very low concentrations (fmol to pmol g\(^{-1}\) of fresh weight) and represent promising targets for improving plant productivity and tolerance to environmental stress factors [139,140].

Phytohormones have been identified in multiple microalgal lineages. Often their functional role remains unclear but increasing evidence suggests that their physiological effects might be similar to those found in higher plants [91]. The research on microalgal phytohormones is still in its infancy but has the potential to reveal attractive perspectives for achieving higher biomass yield and increased productivity of metabolites such as carbohydrates, lipids and proteins. The exogenous application of phytohormones alone or in conjunction with specific abiotic or biotic stress conditions (high salinity, nitrogen or phosphorus depletion, etc.) as well as the genetic engineering of endogenous phytohormone systems are presumably important additional building blocks, which may help to establish microalgal productions at an industrial scale.

3.4.1. Phytohormones to improve the growth rate and carbohydrate accumulation of microalgae

In some microalgae, different phytohormones were shown to induce higher growth rates and increased biomass yields [92], sometimes increased monosaccharides content [141]. For instance, some brassinosteroids (BRs), which are polyhydroxylated steroidal hormones, stimulated increases of two-to-three fold in the growth, and up to three fold in the monosaccharide content, of *Chlorella vulgaris* when used at concentrations of 10\(^{-12}\) and 10\(^{-8}\) mol-L\(^{-1}\) [142]. Also, the growth of *Chlorella vulgaris* in presence of salicylic acid (between 10\(^{-6}\) and 4 \times 10\(^{-4}\) mol-L\(^{-1}\)) increased the cell number and monosaccharide content by up to 40% [143]. The synthetic auxins indomethacin (IM, 10\(^{-7}\) mol-L\(^{-1}\)) and naphthyl-3-acetic acid (NAA, 5 ppm) substantially increased the biomass productivity of two *Chlorella* species (*Chlorella vulgaris, Chlorella sorokiniana*) by about 70–80% on average compared with the control, along with an increased monosaccharide content for IM [144,145]. Additionally, all other types of phytohormones (cytokinins, gibberellins, abscisic acid, ethylene, jasmonates and strigolactones) improved the growth rate of *Chlorella* species [146,151], along with increased monosaccharide/glucose content for cytokinins, jasmonates, and abscisic acid [146,148,150]. In summary, all nine categories of phytohormones have shown improved growth on microalgae species (literature shown for *Chlorella*, but other species showed similar improvements, e.g., [147]) followed in a number of cases by an increase of monosaccharide or glucose production. More research would be needed to identify if cellulose content is also specifically increased by phytohormones supplementation.

3.4.2. Synergistic effects between abiotic stress and phytohormones on the productivity of microalgae

Nitrogen starvation is a useful approach to enhance lipid and carbohydrate production in microalgae. However, the oxidative damage caused by nitrogen depletion dramatically reduces cell growth rates. In a study by Yu et al. [152], a combination of auxin-related phytohormones (indolebutyric acid, IBA; naphthylacetic acid, NAA) together with a nitrogen depletion condition significantly increased cell productivity, increased cell sizes and maintained normal growth rates in *Scenedesmus* and *Chlorella* species. Increased resistance of *Chlorella* to N-stress was also observed for various other phytohormones such as another auxin (Indole-3-acetic acid, IAA), cytokinin (kinetin), and gibberellin (gibberelic acid, GA), resulting in increased biomass productivity in each case [153]. On *Nannochloropsis* species, abscisic acid showed improved growth under nitrogen depletion [154]. This phytohormone also improved the resistance to an excess of salinity applied on *Chlamydomonas* (freshwater species) resulting in a higher growth [155]. Even if this alga probably does not produce cellulose, the finding that phytohormones could improve the resistance to several stress conditions could be relevant to find optimum stress strategies for cellulose production. This area of research deserves more attention.

4. Conversion of algal biomass to cellulose and nanocellulose

Only a limited number of studies have reported the use of algae for cellulose or nanocellulose (CNF, CNC) production (see Table 4). Most of the studies focused on macroalgal species, ranging from Ulvophyceae class such as *Valonia ventricosa, Cladophora glomerata, Boergesenia forbesi*, and *Ulva lataca*. *Posidonia oceanica*, some brown algae (Phaeophyceae) such as *Cystosphaera jacquinotii, Fucus vesiculosus, Laminaria digitata*, as well as red algae (Rodophyta) such as *Gelidium elegans* were also studied for cellulose production. Only Lee et al. and Baba Hamed et al. [22,47] reported the use of microalgae (*Nannochloropsis salina, oceanica* and *gaditana*) for cellulose and CNF production.

The production of CNF from algal biomass involves multiple steps: (i) a purification step consists of breaking down the biomass and separating the cellulose pulp from other impurities; (ii) pre-treatment of the cellulose pulp (mechanical, biological, or chemical) can be used to facilitate the fibre delamination prior to CNF formation and reduce the energy demand linked to CNF production [156,157]; (iii) the last step involves the mechanical disintegration of the fibres where cellulose bundles are completely delaminated to individual fibrils. Alternatively, cellulose nanocrystals (CNC, also called whiskers) can be produced by strong acid hydrolysis (typically around 60% H\(_2\)SO\(_4\) [130]) from purifying cellulose pulp. Usually, CNCs are characterized by their smaller size (2–15 nm wide, 100–500 nm long) compared to CNF (20–50 nm wide, 500–1500 nm long) [158], as well as higher crystallinity [159].

Large-scale production of cellulose (e.g., pulp and paper industry) usually involves lignocellulosic biomass that requires strong delignification processes to break the lignin shell surrounding holocellulose bundles (cellulose and hemicellulose blend) for access. The most common processes entail a combination of aqueous chemical treatment at elevated temperature and pressure (“cooking”) to extract a cellulose pulp, and subsequent bleaching to achieve higher purity (e.g., removal of residual lignin). The chemicals used in these processes usually generate a large number of air and water pollutants, including volatile emissions of chlorine, chloroform, carbon tetrachloride or sulphur, and emissions of chlorinated organic compounds (e.g., dioxins), or chlorophenols in aqueous waste streams [160]. Using algal biomass as an alternative feedstock would ease the purification process since most of the algae species were reported as lignin-free [161], thus avoiding the need for strong “cooking” processes and possibly limiting the use of bleach. The purification step for algae serves mainly for removal of hemicelluloses, other polysaccharides such as pectins or lipidic fractions. Some algae species were reported to contain a certain proportion of lignin (e.g., *Posidonia sp., Ulva sp.*). In that case, softer cooking and/or bleaching might be required. In general, non-wood species are easier to cook than wood chips, therefore conventional Kraft pulping can be replaced by soda cooking (sodium hydroxide only), thus reducing the...
quantity of pollutants produced [160].

Very little is known about the influence of the algal cellulose structure on subsequent CNF formation. As mentioned previously, the geometry of the TCs has an influence on the cellulose microfibril structure, which changes depending on the algae species. Many algal species differ from the classical rosette structure found in lignocellulosic biomass. Some specific microalgae such as *Nannochloropsis* sp. were found to have a porous structure of thin cellulose microfibrils [37], which makes the delamination process much easier compared to lignocellulosic biomass [22]. In that case, CNF can be produced without any mechanical treatment, thus significantly reducing the energy consumption linked to CNF production. It would be worthwhile to investigate other microalgae candidates, which could be a very promising feedstock for CNF production if they exhibit similar “easy to delaminate” cellulose substructure. Coupled with its fast growth, decent cellulose content (at least for *Nannochloropsis* sp.), and absence of lignin, microalgae could be a serious competitor to wood-based CNF.

4.1. Cellulose purification

Most of the cellulose purification treatment reported for algae consisted of a combination of bleach (mainly chlorite), alkaline hydrolysis (mainly sodium hydroxide), and dilute acid hydrolysis (hydrochloric acid), in some cases with additional hot water extraction (Table 4).

| Algae strains | Cellulose purification | Conversion to CNF: pre-treatment (biological or chemical) | Conversion to CNF: main treatment (mechanical) | Conversion to CNC: chemical treatment | References |
|---------------|------------------------|----------------------------------------------------------|------------------------------------------------|-------------------------------------|------------|
| *Nannochloropsis salina*, *N. oceanica* | Ethanol hexane, Sodium hydroxide, Sodium chlorite | TEMPO oxidation | – | – | [22] |
| *Nannochloropsis gaditana* | Ethanol toluene, Sodium hydroxide, Sodium chlorite | – | – | – | [47] |
| *Posidonia oceanica* | Ethanol toluene, Sodium hydroxide, Sodium chlorite, Potassium hydroxide | – | – | Hydrochloric acid | [20] |
| Ulva lactuca | Ethanol, Ammonium oxalate, Sodium hydroxide, Hydrochloric acid | – | – | – | [56] |
| Cladophora sp., *Valonia ventricosa*, *Boergesenia forbesii* | Sodium hydroxide, Hydrochloric acid or Potassium hydroxide, Sodium chlorite | – | – | Hydrochloric acid or Sulfuric acid | [57] |
| Cladophora glomerata | Sodium chloride, Sodium hydroxide, Hydrochloric acid | Enzymatic hydrolysis | Blending, Micro-fluidization | – | [55] |
| Cladophora sp. | Sodium hydroxide, Hydrochloric acid | – | – | – | [165] |
| Valonia ventricosa | Sodium hydroxide, Hydrochloric acid | – | – | – | [166] |
| Cystosphaera jaccquiniottii | Sodium hydroxide, Hydrochloric acid | – | – | – | [71] |
| Fucus vesiculosus, Laminaria digitata | Sodium hydroxide, Sodium carbonate, Hot water | Supercritical fluid, Hydrochloric acid | – | – | [72] |
| Gelidium elegans | Ethanol toluene, Sodium hydroxide, Hydrogen peroxide | – | – | Sulfuric acid | [73] |
| Red algae waste (from agar production) | Sodium hydroxide, Sodium chlorite | – | – | Sulfuric acid, Ultra-sonication | [19] |
Solvant-based (ethanol, ethanol-hexane, ethanol-toluene) or supercritical fluid extraction was also occasionally reported as techniques for lipid extraction.

The cellulose purification of *Nannochloropsis* species was reported as a three-step process: lipid extraction, sodium hydroxide treatment and bleaching. Ethanol/hexane (1:1, v/v) or ethanol/toluene (32:68, v/v) is used for the defatting step, the lipid-free biomass is further treated by 2% or 4% NaOH at 80 °C for 2 h to remove the hemicellulose, followed by bleaching at 70 °C in acetate buffer (pH 4.8–4.9) using 1.7% NaClO2 for 4 h or 6% to 10% NaClO2 for 2 h [22,47]. As no lignin is expected in *Nannochloropsis* sp., the bleaching step could be used to remove the pigments. However, a high concentration of bleach could have a negative effect on the crystalline structure of cellulose. For instance, a 10% NaClO2 concentration was reported to create more amorphous sites on the semi-crystalline cellulose structure [47].

For macroalgae species, a broader range of processes were proposed for cellulose purification. High lignin content species such as *Posidonia oceanica* (about 30% lignin) needed strong delignification process to obtain purified cellulose pulp. Bettaieb et al. [21] proposed a three-step procedure: (i) biomass delignification using soda-anthraquinone (Soda-AQ) treatment (20% NaOH and 0.1% anthraquinone) for 2 h at a temperature ranging between 150 °C and 170 °C, (ii) bleaching with 12% NaClO for 15 min at pH 12, (iii) alkaline treatment to remove hemicellulose using 4% NaOH at 80 °C for 2 h. Usually, alkaline hydrolysis treatment can remove lignin and a significant part of hemicellulose [162], here anthraquinone was added to NaOH to catalyse delignification [163]. Similarly, Tarchoun et al. [20] reported a softer treatment on milled dried *Posidonia* (i) dewaxing using ethanol/toluene (1:2, v/v) treatment, (ii) hot water extraction for 5 h to remove sugars, colouring matter, and starch, (iii) bleaching at 70 °C using 1.7% NaClO in acetic acid solution (pH 3–4) for 1 h to remove lignin (the process was repeated 7-times), (iv) 5% KOH treatment at room temperature for 24 h and 90 °C for 2 h to remove hemicelluloses and residual pectin. It should be noted that at concentration beyond 8% to 9% of NaOH, cellulose mercerization occurs which leads to irreversible conversion of the cellulose structure from native cellulose I to cellulose II [164]. However, this can be beneficial for further pre-treatment as the hydrogen-bond becomes disordered (swelling), resulting in increasing the accessibility of chemicals or enzymes between the fibres.

Lower lignin content macroalgae such as *Ulva lactuca* (about 10% lignin reported [66]) could also be purified under similar bleaching conditions than the soft treatment for *Posidonia oceanica*. After lipids and extractives removal (ethanol treatment), and ulvan extraction using ammonium oxalate, the biomass was bleached at 60 °C using 2% NaClO2 in 5% acetic acid solution until biomass discouloured and turned white. An alternative strategy for hemicellulose extraction was reported here using 0.2% NaOH at 60 °C treated overnight, followed by boiling at 5% HCl, then at 30 °C [56].

High hemicellulose content in macroalgae such as that found in red algae *Gelidiella elegans* (about 30% hemicellulose [66]) was also purified under similar alkali conditions as reported above. A 2% NaOH treatment at 80 °C for 2 h was able to decrease hemicellulose from 29.5 to 8.2% while decreasing the lignin content slightly (from 4.5 to 3.4%). An alternative bleach treatment using hydrogen peroxide (30% H2O2 at 80 °C for 1.5 h, twice) was used afterwards to remove residual hemicellulose and lignin, (decreasing to 2.0% and 0.7% respectively) showing the efficiency of the combination between alkali treatment and bleach for purification.

### 4.2. Production of cellulose nanocrystals

After cellulose purification, cellulose nanocrystals were sometimes produced using concentrated hydrochloric or sulfuric acid. Imai et al. and Tarchoun et al. [20,57] reported the production of algal CNC using 9% HCl solution boiled from 30 min to 12 h or using 40% H2SO4 solution mixed at 70 °C for 3 days on cellulose pulp originating from *Valonia*, *Cladophora*, *Boergesenia* and *Posidonia*. Stronger hydrolysis conditions were reported by Bettaieb et al. and Kasab et al. [19,21] on pulp from *Posidonia oceanica* and red algae waste using 64% H2SO4 at 55 °C for 20 to 40 min. The CNC was further sonicated for 5 min for reduction of aggregate size. The crystallinity increased after the acid treatment and the aspect ratio of the CNC was unsurprisingly low with an average diameter of the nanocrystals of ≈10 nm and length of ≈300 nm.

### 4.3. Production of cellulose nanofibrils

Algal CNF can also be produced from previously purified cellulose pulp. Xiang et al. [55] achieved successful CNF production from *Cladophora glomerata* using a combination of enzymatic hydrolysis (cellulase enzyme blend containing cellulases, β-glucosidases, and hemicellulases) and micro-fluidization. While cellulases helps to liberate individual fibrils by hydrolysing the amorphous region of cellulose (endoglucanases) or attacking the reducing and non-reducing ends of cellulose chains to produce tetra- and di-saccharides (cellulobiohydrolases), the β-glucosidases further hydrolyses the tetra- and di-saccharides to glucose [168]. It was also shown that other enzymes such as hemicellulases (ylanase), laccases, and lytic polysaccharide monoxygenases (LPMO) increases cellulose accessibility to cellulase enzymes and improves fibre delamination [169–171]. In the case of Xiang et al. [55], a 2% (w/v) cellulose suspension was mixed in a sodium acetate buffer at pH 4.8 with an enzyme charge of 3 FPU g−1 glucan, the solution was incubated at 50 °C and shaken at 150 rpm between 1 h to 48 h. Cellulose samples with and without enzymatic treatment were soaked in water overnight before disintegration in a blender for 30 s at 30000 rpm. The suspension diluted to 0.2 wt% was then passed 10 to 20-times in a 200 μm microfluidizer chamber and an additional 10 to 20-times in an 87 μm chamber. This produced fibrils of 10–40 nm diameter and several μm in length with a crystallinity ranging between 80% and 85% which showed that the pre-treatment decreased the crystallinity, but had little change to the diameter distribution. However, the use of endoglucanase, exogluclanase and β-glucosidases blend is questionable since it was shown that endoglucanase alone helped reduce depolymerization compared to multi-enzyme blend while producing a better separation of the nanofibrils [172].

Hanley et al. [58] proposed to isolate cellulose microfibrils from previously purified cell wall of *Valonia ventricosa* by a combination of strong acid hydrolysis (67% H2SO4 at room temperature for 30 min and 70 °C for another 30 min) and ultra-sonication (2 min on a 1 w% suspension). This may be surprising since strong acid hydrolysis would tend to produce nanocrystals rather than nanofibrils. However, this treatment was found to isolate fibrils of several μm in length and 7–12 nm in width, which is closer to CNC than CNF. This result could be explained by the extraordinary resistance of *Valonia* cell wall to acidic treatments as it appeared that in 72% sulfuric acid, the cell wall does not swell and the microfibrils are hardly affected [65]. In that case, the residence time was much less (1 h) than CNC production from *Valonia* sp. reported above (3 days), showing the decrease of treatment severity.

Lee et al. [22] showed that it was possible to produce CNC from microalgae (*Nannochloropsis* sp.) without any mechanical treatment to drastically reduce the energy consumption usually needed for the mechanical shearing of the cellulose pulp. The previously purified cellulose slurry was subjected to TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) oxidation to enhance delamination to individual fibril. TEMPO oxidation is one among many chemical pre-treatments that could be used to introduce repulsive ionic charges between elementary cellulose fibres, and help their delamination by weakening the cohesion of hydrogen bonds between the fibres [158,160]. Chemical pre-treatments can tune cellulose with new properties by functionalization of its surface and produce a unique grade of CNC [168], TEMPO/NaBr/NaClO was mixed (gently) to the cellulose pulp for 2 h, while the pH was maintained at 10 using a 2% NaOH solution [22]. The isolated fibrils were 1–9 μm and about 10 nm wide with an exceptionally high crystallinity (91.7%). It
was suggested that the efficient fibrillation to CNF (without mechanical treatment) could be due to two factors: (i) the material was never dried during the process which helps to avoid tight agglomeration of cellulose fibres through hydrogen bonding and allows reagents to find space between the fibrils, (ii) the cellulose layer of Nannochloropsis sp. has a delicate fibrous substructure (fluffy and porous) composed of thin fibrils compared to the one of red algae and land plants arranged through linear rows or rosettes, so such structure was found in Nannochloropsis sp. [22,37]. Further research is needed to understand if other pre-treatments would lead to the same efficiency of delamination. For TEMPO oxidation, one improvement could be to use TEMPO/NaClO/NaClO₂ instead of TEMPO/NaBr/NaClO as the former has been reported to overcome cellulose depolymerization [173,174]. However, the use of bleaching agents such as NaClO or NaClO₂ should be avoided if possible due to their toxic potential. Additionally, the chemicals generally used for TEMPO oxidation are difficult to recover and expensive [158]. Other cellulose functionalization (chemical pre-treatment) or pre-treatment without functionalization (e.g., enzymatic hydrolysis) should be tested to find more cost-effective and environmentally benign solutions of CNF production from Nannochloropsis sp.

4.4. Future opportunities for algal nanocellulose production

The current literature on the production of cellulose or nanocellulose (CNF or CNC) from algae is very scarce. Herein, we propose a bigger picture of the processes that can be used to produce CNF or CNC from algal biomass to identify future opportunities (Fig. 6). It should be noted that by changing the combination of cellulose purification processes, pre-treatment and mechanical treatment, various grades of CNF with unique properties (fibril morphology, crystallinity, degree of polymerization, etc.) can be obtained from the same biomass [168]. Post-treatment could also be added afterwards to functionalize CNF with specific properties (modification by adsorption, molecular or polymer grafting) [158,168] or fractionate the nanofibrils (centrifugation, filtration) [168].

![Fig. 6. Possible conversion processes from algal biomass to nanocellulose materials. A first purification step is required to separate cellulose from the other components of the biomass. A combination of several purification treatments can be considered depending on the biomass composition. In a second step, the cellulose can be converted to cellulose nanocrystals (CNC) or cellulose nanofibrils (CNF). A large variety of processes is available for CNF conversion. Usually the mechanical treatment can be sufficient to achieve cellulose fibrillation and CNF formation, however a pre-treatment step (biological, chemical, mechanical) can facilitate the fibrillation or in some specific cases directly achieve total fibrillation to CNF.](image)

4.4.1. Cellulose purification

The literature on algal cellulose production revealed that a combination between alkaline hydrolysis (NaOH, sometimes KOH) and bleach (NaClO₂, sometimes NaClO), with potential additional solvent-based lipid extractions, was efficient in most cases to extract a purified cellulose pulp from raw biomass. However, comparative studies of the best purification procedure for specific algae species is still lacking. It might be worth trying other processes to see if the use of a bleaching agent can be avoided or limited. For instance, dilute acid hydrolysis or hot water extraction was not often reported. Dilute acid hydrolysis (typically <4 wt%) using various mineral or organic acids at elevated temperature was shown to efficiently hydrolyse hemicellulose [175], while more severe conditions (higher temperature or concentration) tend to hydrolyse cellulose to individual glucose units [176]. Liquid hot water treatment was also shown to cause significant hemicellulose solubilisation and partial lignin degradation, while possessing the advantage of being chemical-free [162]. Other selective solvent-based purification processes could also be used for algal biomass fractionation. They include for instance organosolvation and ionic liquids. Organosolvation can involve a variety of organic or aqueous-organic solvent mixture (with or without acid catalyst) to efficiently remove hemicellulose and lignin (if necessary) [175], while ionic liquids are able to specifically remove certain components (e.g., lignin) depending on the solvent used [162].

4.4.2. Production of cellulose nanofibrils

The production of algal CNF reported only the classical processes that are generally used for nanofibrillation, biological (enzymatic) pre-treatment, classical chemical pre-treatment (TEMPO oxidation) [158], and mechanical treatment (high-pressure homogenization, in that case with a microfluidizer) [168]. In addition to the fact that it would be interesting to test other algae species using these conventional processes, it also seems relevant to test other pre-treatment or mechanical treatment processes to produce CNF with unique properties.

The available chemical pre-treatments can be separated in two main...
categories: (i) modification of cellulose hydroxyls groups, (ii) opening of the cellulose ring and introducing the aldehyde groups (dialdehyde cellulose) which are then modified to other functional groups [158]. Hydroxyl groups can be modified to carboxyl (TEMPO oxidation) [174,177,178], carboxymethyl (carboxymethylation) [179,180], sulfonate (sulfoethylation) [181], and phosphoryl (phosphorylation) groups [182,183], or by introduction of cationic ammonium sites (cationization) [184–186]. For the ring-opening methods, periodate oxidation can be used to cut the C–C bond between C2 and C3 positions on the cellulose ring to form aldehyde groups, which can be turned to alcohol [187], carboxyl [157,188] and sulphite groups [189,190], or cationic ammonium [191,192]. Ozonation has also been reported to be a cheap method to introduce carboxyl groups [193]. Soft mechanical treatment could also be used as pre-treatment in addition to biological or chemical pre-treatment or as a unique pre-treatment step. This includes refining, blending, and grinding [194–197].

The mechanical treatments generally achieve total fibrillation to individual cellulose fibres (i.e., CNF) using high shear force processes. Conventional processes include high pressure homogenization (homogenizer, microfluidizer) [198,199], ultra-fine friction grinding [200] and refining [201]. These processes are considered to be the most efficient to delaminate cellulose bundles for CNF production, and are suitable for upscalling [168]. Other emerging processes, as shown in Fig. 6, can also be used for mechanical disruption [202–208].

5. Past, current and future obstacles in End of Life scenarios linked to bioplastic

This section provides some insight into the current research activities and state of the art in relation to the possibilities and the difficulties encountered towards the various end of life scenarios of bioplastic materials (Fig. 7). Although it is not directly linked to the production of CNF from algae and materials produced from it, the discussion herein is necessary to identify, name, and ultimately prevent the issues evolving currently within this domain. It is understood that highlighting the present situation will ensure that algal-based materials do not suffer from the same uncertain end of life scenarios as current bioplastics from other sources.

Alternatives to petro-based plastics are primarily wanted by policymakers, requested by conscientious customers, developed by researchers, commercialised by industry and praised by most as a “greener and better” solution. However, there remains the problem of whether these new products offer the complete consumer experience (and expectations) as advertised by industry, in particular pertaining to biodegradability and compostability promised by producers as a superior alternative to current non-renewable material [209–211]. Traditionally, industry had focused predominantly on the development of a stable, long-lasting product capable of sealing perishable goods and increasing their edibility duration [212]. Recently, the focus has shifted towards improving the material life cycle to balance the need for quality as well as the product specification of plastics produced from renewable biomass [213]. In addition to this, the biodegradability, compostability, benignity towards animals and environment is also a metric considered towards the progressive shift, as pointed out by Sidek et al. [213]. Thus, novel materials are required to meet the established international material standards tested by official certification bodies (e.g., DIN CERTCO (Germany) TÜV Austria (Austria), etc.) in order to guarantee a uniform certification representative of the standard specified. These standards are based on conventional packaging material assessments which are recoverable through composting and biodegradation, e.g., EN 13432. Unfortunately, the scientific results show that the established industry standards for such certifications might not be fully applicable to degradable bioplastics. They do not project a realistic lifetime and therefore have to be adapted, as stipulated by Harrison et al. [214], Van den Oever et al. and Haider et al. [215,216] address the issue to investigate the special certification requirements for biodegradable plastics, as well as present drawback and improvement potential of present testing scenarios. Haider et al. point out “the necessity for tests under environmentally authentic and relevant field-testing conditions”, thus, confirming that the current standard certifications do not embody realistic parameters.

Furthermore, Napper et al. [217] showed the low degradability of biodegradable certified plastic shopping bags, but with non-official labels. The study led to widespread media attention, potentially resulting in a loss of consumer confidence in biodegradable materials as shown by Blesin et al. [218]. These findings raise another important question as to why uncertified and unsuitable bioplastics receive approval for distribution to consumers.

![End of Life scenarios](image_url)

**Fig. 7.** Possible End of Life scenarios for bioplastic materials.
Standard protocols like DIN ISO 4892 [219,220] exist to assess and indicate the lifespan of plastic materials under accelerated weathering conditions. Friedrich et al. [221] showed that by using Wood-Plastic-Composite in accelerated weathering tests and under real conditions, there was no correlation between results obtained from laboratory experiments and under real conditions. Shimizu et al. [222] reached a similar conclusion while trying to link polypropylene degradation from accelerated to natural weathering, but indicated that the carbonyl index is an applicable measure. Different locations and therefore varying parameters like yearly rainfall, average temperature, soil pH and structure, aeration, prevailing enzymes, humidity etc. could have a major impact on the degradation process, but these cannot be fully replicated in a laboratory environment, and thus require field trials at various locations [223,224]. Additionally, the accumulation of plastic residues in soil can result in poor harvest as well as affect the local wildlife. Hegan et al. [225] investigated the degree of annual film mulching of cotton fields, where a clear trend was detected showing a constant decrease in cotton yield from accumulation of plastic material during harvest cycles over the years.

According to Van den Oever et al. [226], composting is currently the best solution to fully decompose biodegradable plastic material, followed by recycling and/or landfilling. Kern et al. [227] tested composting bag materials “in-situ” at four German composting facilities, and showed that all tested materials decompose visually within the standard process time of the facility but without analysing the chemical debris of the degradation process. Even if the permitted proportion of materials other than biomass (e.g., additives, plasticizers) in bioplastics is below 10 wt%, they can still pose a detrimental affect in the compost as well as the soil, potentially resulting in negative effects on microbial life, plant growth [228,229], or even human health [230].

As such, the question arises as to what is really happening to all the additives and plasticizers once the material decomposes. In particular, monitoring and determining degradation in aqueous environments is especially challenging given the variations and conditions involved. It could be suggested that this is primarily due to the lack of experience in open environment degradation, but could also be from the lack of established protocols and analytical methods to assess the whole end of life. Approaches have been made to identify suitable analytical methods but the nature of biodegradable material presents a special challenge to laboratory equipment and procedures [231,232]. Even though laboratory tests lead to reliable and reproducible results in a controlled environment, the outcomes do not represent reality as the conditions are not sufficiently simulated [233]. Hence, the results are not transferable to real-life scenarios as weathering under natural environments and the material life and consequences cannot be precisely projected for various reasons, which is also mentioned in the international standard as pinpointed by Crewdson [234].

Ultimately, the main research question that enables the circularity of new products and innovative processes should also be based on questions such as: what is the true End of Life scenario for bioplastics currently? Can it be properly recycled and reintegrated into the supply chain without using new (or minimal) materials coming from renewable resources or will it ultimately complicate conventional plastic recycling once it is introduced to the recycling stream? Van den Oever et al. [215] showed that biomaterials in conventional plastic recycling stream had very little problems for actual recyclability. However, concerns remain about bioplastic materials to which chemicals are added to increase the desired material properties. These will eventually create problems in the long run [229], even though research is not yet able to identify, quantify and name all the end products sufficiently.

6. Life cycle assessment of algal bioplastic

It should be noted that computation of the life cycle assessment (LCA) is very much dependent, but not limited to, the system boundary, products considered as well process variables and route. Past research heavily focused on the cultivation steps where a tradeoff between high productivity (and control) in a photobioreactor (PBR) versus a lower energy input and environmental footprint in an open-pond reactor provided interesting results. However, very limited amount of research exists on algae bioplastics [235], as most of the focus has thus far been on wastewater water treatment using algae for biofuels production.

To this effect, the most relevant study towards developing an LCA for algae-based bioplastics to date was conducted by Beckstrom et al. [236]. Based on cultivation in an open pond reactor (OPR), circular flow photobioreactor (CF-PBR) and combined OPR/CF-PBR, as well as three processing routes entailing fractionation, lipid extraction and drying only, bioplastic feedstock preparation was considered within the system boundary. Under different scenarios, the most promising bioplastic feedstock preparation route utilised the CF-PBR. The emissions associated with the CF-PBR and OPR accounted for ~0.315 and 0.656 kg CO₂ eq/kg bioplastic feedstock via drying and fractionation processing routes, respectively. The negative emissions accounted mostly from the presence of biogenic CO₂ from the atmosphere during cultivation. However, this credit was eroded via the fractionation route since one of the by-products was fuel, resulting in emissions during its combustions. It is well understood that in order to truly support advancement of algae as a source of commodity chemical, material or fuel, the vision of an algal biorefinery should be realised. To maximise sustainability and commercial viability, such a process would be expected to create multiple products, which could in theory also be fuels. As presented in the study above, this could in fact make the algal to bioplastics vision environmentally susceptible. It is known that resins such as nylon and polypropylene hold environmental burdens of 8.03 and 1.98 kg CO₂ eq/kg bioplastic [237], respectively, which is approximately three times greater than that computed by Beckstrom et al. [236]. It could be argued that the environmental burdens associated with algal processing can be improved via the use of renewable energy sources in the energy input stream, but such a hypothesis is not unambiguous [238].

Further to this, any bioplastic material designed with a view of sustainability should consider biodegradation. During this process, there will certainly be an aspect of CO₂ being released to the atmosphere once the material starts to decompose. Thus, any positive effects computed during the bioplastic resin manufacturing boundary would be lost as the material reaches its grave. Such cradle-to-grave approach as opposed to cradle-to-gate or other such variant can yield misaligned environmental footprints, or even not represent the hotspots appropriately. Especially, since the end-of-life aspect as well as the burden associated with supply chain remains unattended. Thus, an important point to consider is the reliability of the results presented from LCAs carried out in various studies. Not only are there substantial differences from factors mentioned above, but there are also inconsistencies present due to the standard methodology applied to obtain the environmental burdens and/or data-sets for biopolymers in the inventory [239]. Considering the limited number of investigations currently available in the algal bioplastics field, it could be worth bearing in mind that a standardised methodology to ensure credibility and ease of comparison of the burdens is respected. As such, Ogmundarson et al. [240] recommended the ISO 14040 standard series as a benchmark. Similarly, ISO 14044 could also prove to be a successful means towards harmonizing results from future studies since they are both based on following a cradle-to-grave approach.

7. Conclusion

The vision of an algal biorefinery offers a promising alternative to the traditional petro-chemical industry. Although several algal products have now been commercialised at scale (nutraceuticals, value products, etc.), the complete concept suggested by many researchers has not yet been reached. At present, research on algal cellulose is limited, but rapidly growing, and the desirability of species with high purity and quantity is much sought after. Stress-induced methods to increase
cellulose production biologically can be achieved, but this usually comes at the expense of the expense of productivity. To that effect, genetic engineering techniques such as gene editing or recombinant DNA could be beneficial, provided the regulatory organizations and governments allow modified species to be used commercially. Bioplastics are an important and crucial element in this puzzle with demand in line with quantities of petro-fuels. Thus, the infancy of the algal bioplastic field has to be overcome as there is already a pull factor from the market resulting in producers/users finally pushing for commercialisation. The costs remain uncertain given the extraction and conversion process of cellulose when applied to algae, particularly for technologies that are translated from existing biomass feedstock. However, the technical understanding, research and development as well as prototypes to showcase algae’s potential is severely lacking and has to be recognised as a bottleneck. To close the material loop for bioplastics (and realise an algal biorefinery), it is of utmost importance to collaborate within and beyond the technical fields to produce a product that is at the forefront of innovation, both in sustainability and acceptability. Whether the algal bioplastics vision will push forward the algal field into the commercial realm or prove to be another potential by-product with limited economic/environmental credentials remains to be seen.

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