Algae biostimulants: A critical look at microalgal biostimulants for sustainable agricultural practices

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Abstract

For the growing human population to be sustained during present climatic changes, enhanced quality and quantity of crops are essential to enable food security worldwide. The current consensus is that we need to make a transition from a petroleum-based to a bio-based economy via the development of a sustainable circular economy and biorefinery approaches. Both macroalgae (seaweeds) and microalgae have been long considered a rich source of plant biostimulants with an attractive business opportunity in agronomy and agro-industries. To date, macroalgae biostimulants have been well explored. In contrast, microalgal biostimulants whilst known to have positive effects on development, growth and yields of crops, their commercial implementation is constrained by lack of research and cost of production. The present review highlights the current knowledge on potential biostimulatory compounds, key sources and their quantitative information from algae. Specifically, we provide an overview on the prospects of microalgal biostimulants to advance crop production and quality. Key aspects such as specific biostimulant effects caused by extracts of microalgae, feasibility and potential of co-cultures and later co-application with other biostimulants/biofertilizers are highlighted. An overview of the current knowledge, recent advances and achievements on extraction techniques, application type, application timing, current market and regulatory aspects are also discussed. Moreover, aspects involved in circular economy and biorefinery approaches are also covered, such as: integration of waste resources and implementation of high-throughput phenotyping and -omics tools in isolating novel strains, exploring synergistic interactions and illustrating the underlying mode of microalgal biostimulant action. Overall, this review highlights the current and future potential of microalgal biostimulants, algal biochemical components behind these traits and finally bottlenecks and prospects involved in the successful commercialisation of microalgal biostimulants for sustainable agricultural practices.

Keywords Algae biostimulants, sustainable agriculture, microalgae biotechnology, metabolomics, consortia, biorefinery, circular economy, bioremediation

1. Introduction

Modern agricultural practices and future perspectives for agro-industries are of current relevance, and are faced with two challenges: i) improving crops quality and yield as a result of growing world population, whilst preventing losses due to biotic and abiotic stresses which are currently 30-40% and 60-70% respectively and ii) minimising the impact on the environment and human health caused by the widespread use of mineral fertilisers and chemical products intended for improving crop quality and yields (Colla et al., 2017; Win et al., 2018). In addition, the forthcoming regulatory framework is restricting the use of such chemical inputs due to the growing demand for organic food and rising environmental awareness (Chiaiese et al., 2018; Dmytryk and Chojnacka, 2018; Mzibra et al., 2020). This is further complicated by issues such as reaching the genetic potential of stable crops and decreasing areas of fertile land caused by urbanisation, erosion and adverse effects of climate change, forcing farmers worldwide to produce more with less (Povero et al., 2016; Shukla et al., 2019). Biofertilizers and biostimulants have the potential to mitigate these issues and provide a renewable option for improving crop quality and yield. Biofertilizers are environmentally friendly resources containing microorganisms, such as bacteria, fungi or microalgae, that promote plant growth and development by colonising the rhizosphere of the plant and allowing increased absorption of nitrogen, phosphorus, potassium and minerals and are applied as large volumes (Bhattacharjee and Dey, 2014; Win et al., 2018). Whereas, biostimulants are resources that, when applied in small quantities, enhance plant physiological processes resulting in improved crop nutrition, stress tolerance, yield or quality without causing damage to, or better still improving, the surrounding environment (Arnau and Richard, 2016; Barone et al., 2018; Kopta et al., 2018). According to the European Biostimulants Industry Council (EBIC), biostimulants increase nutrient use efficiency and help plants tolerate abiotic stresses which both enhance the quality and yield of crops (EBIC, 2016). It is important to note that biostimulants are not biofertilizers as they do not provide nutrients to the crops directly, instead they facilitate the nutrient uptake by modifying the rhizosphere and plant metabolism resulting in enhancing nutrient efficiency, tolerance to abiotic stresses, and improved crop quality (Drobek et al., 2019). The main categories of plant biostimulants are humic substances (humic acid, fulvic acids, and humins); algae extracts; protein hydrolysates (signalling peptides and free amino acids) and microorganisms (bacteria, yeast, filamentous fungi, and microalgae) (Bulgari et al., 2019). Alternatively, they can be simply classified as

microbial and non-microbial plant biostimulants, where, macroalgae extracts, humic substances and protein hydrolysates fall within the non-microbial category (Rouphael and Colla, 2020).

There are nine objectives of the EU common agricultural policy post-2020 which include protecting the quality of food and health, preserving landscapes and biodiversity, caring for the environment and taking action on climate change ("Eur. Comm.," 2018). In this context, interest in biostimulants is growing and the use of algal extracts for this purpose is becoming more popular (Michalak et al., 2015). Research has shown that algae (both microalgae and macroalgae) have biostimulant potential to promote overall plant development and growth along with improved tolerance to abiotic stresses (Blanke, 2016; Craigie, 2011; Crouch and Van Staden, 1993; Michalak et al., 2017; Sharma et al., 2014). A wide range of biostimulatory compounds have been identified in macroalgae extracts including amino acids, polysaccharides, vitamins, fatty acids, mineral elements, phenolics and traces of phytohormones. Macroalgae have been largely exploited since the early 1980s for their biostimulant potential and represent a key category within the organic plant biostimulant market. The macroalgal biostimulant market is more established compared to the microalgae. However, due to the diverse composition and physicochemical properties of the bioactives, the specific underlying mode of biostimulant action of individual bioactives is often unclear and still under investigation. Recent biotechnological advancements such as high-throughput phenotyping and -omic platforms are proving to be a potential solution in illustrating the underlying modes of biostimulant action and will subsequently enable the development of novel products (Bulgari et al., 2019). There is still substantial scope in enhancing the biostimulant and commercial potential of macroalgae extracts which has been extensively reviewed in recent times (Bulgari et al., 2019; EL Boukhari et al., 2020; Nabti et al., 2017; Sharma et al., 2014; Shukla et al., 2019) and hence it will be beyond the scope of this review.

Microalgae are a diverse group of mainly single celled photosynthetic organisms that use sunlight and CO_2 to synthesise a wide range of metabolites. To date, microalgae have been explored for use in the fields of biofuels, aquaculture and animal feeds, bioremediation of waste, nutraceuticals, pharmaceuticals and cosmeceuticals (Chanda et al., 2019). In the case of agricultural applications, microalgae have been explored less in comparison to macroalgae. Traditionally, cyanobacteria (blue-green algae) are known for their nitrogen-fixing capacity (~20-30 kg N/ha) in paddy fields and are beneficial for a variety of other crops (Chakdar et al., 2012). Soil application of harvested microalgal biomass is known to act as a slow-releasing biofertilizer and soil conditioner (Dasgan et al., 2012; Garcia-Gonzalez and Sommerfeld, 2016). Whereas, application of living cyanobacteria is known to act as a potential biocontrol agent, via activation of plant defence enzymes and production of hydrolytic enzymes and antimicrobial compounds, against plant pathogens (Gupta et al., 2013).

Compared to macroalgae, research into microalgal biostimulants is becoming more prevalent due to numerous reasons. For example, acquisition of a reliable source of biomass is needed to gain economic viability, however, standardisation and reliability of macroalgae extract is unsustainable and difficult to govern as currently most of the seaweed is wild-harvested and its biochemical composition is hampered by seasonal and climatic changes. Also, the harvest of seaweed is strictly regulated in some countries to prevent unsustainable practices which could cause the collapse of ecosystems and therefore loss of supply, however, this means that most of the biodiversity remains untapped (Battacharyya et al., 2015; EL Boukhari et al., 2020; Sharma et al., 2014). In contrast, microalgae offer a sustainable platform as a renewable source of biostimulant as the quality of extract can be more easily governed under controlled conditions and tailored to produce specific bioactives. Moreover, research comparing both macroalgal and microalgal biostimulants suggest similar biostimulant activity, for example, fruit production of tomato plants was found to be similar when using either micro or macroalgae as biostimulants (Oancea et al., 2013). Henceforth, microalgae could be a viable sustainable source of bioproducts for the development of plant biostimulants.

Furthermore, microalgae are reported to be a sustainable alternative for enhancing and protecting crops, with the additional benefit of improving soil quality by regenerating microbiological interactions (Arnau and Richard, 2016; Garcia-Gonzalez and Sommerfeld, 2016). However, an extensive application has not been established and microalgae are yet to be fully exploited for their use as plant biostimulants. Several reports have demonstrated the potential biostimulant activity of microalgal extracts on a variety of crops (Barone et al., 2018; Dias et al., 2016; Garcia-Gonzalez and Sommerfeld, 2016; Guedes et al., 2018; Oancea et al., 2013; Plaza et al.,

2018). Moreover, development of microalgal biostimulants via integration of waste nutrients such as wastewater (Renuka et al., 2017) and anaerobic digestion waste (digestate) (Stiles et al., 2018) as a free nutrient input offers unique opportunities in shaping circular economy platforms. A recent literature survey on the highest scientific trends and market opportunities analysis identified microalgal bioplastics and biostimulants as the major emerging concepts (Rumin et al., 2020). However, a major challenge remains in unravelling the mode of biostimulant action of specific bioactives due to variability of algal and crop species and their interactions depending on abiotic factors (Bulgari et al., 2019). Nonetheless, the increasing cost of chemical fertilisers, pesticide resistance and effects of climate change, when considered alongside the promising capabilities of microalgal biostimulants, it is clear that microalgae have an immense scope, and provide a major opportunity to make agriculture more sustainable and resilient.

We present a thorough review discussing the current knowledge on potential algal biostimulants, their key sources and available quantitative information. Biostimulants covered include phytohormones, proteins, amino acids, humic acids, fulvic acids, polysaccharides, antioxidants, vitamins and enzymes. While the review critically evaluates the best way forward for the microalgal biostimulant industry, the physiological effects and quantitative information on already established macroalgal biostimulants is also included for comparison purposes. The prospects of microalgal biostimulants to advance crop production and quality is highlighted, where aspects covered include specific biostimulant effects caused by extracts of microalgae, microalgal consortia and microalgal-bacterial consortia, extraction techniques, application type-timing, current market and regulatory perspective. Other key aspects covered to optimise across the supply chain include the integration of waste resources and high-throughput phenotyping/-omics approaches for the development of sustainable circular economy/biorefinery approaches.

2. Specific biostimulant effects of microalgae and consortia on production and quality of crops.

2.1 Biostimulant effects caused by microalgae

Root and foliar application of the cyanobacterium, *Arthrospira platensis*, at different concentrations have been evaluated for their biostimulant activity on papaya. After inputting the results into a surface model for a number of leaves, stem diameter, plant height and leaf area, it was concluded that 1.08% (*w/v*) root application was optimal for biomass production of the papaya seedlings, whereas no effect was observed when the same suspension was foliar sprayed onto papaya plants (Guedes et al., 2018). Foliar application of the cyanobacteria *Anabaena vaginicola* or *Nostoc calcicola* on cucumber, squash and tomato plants caused significant increases in dry weight, fresh weight, root length, height and leaf number compared to controls for all three plant species (Shariatmadari et al., 2013). *A. platensis* biomass was evaluated as an organic biofertilizer and compared to an NPK fertiliser for the cultivation of sweet pepper in sandy soils (Aly and Esawy, 2008). The *A. platensis* had 6.7% N, 2.47% P, 1.14% K and the NPK fertiliser had recommended doses of 15.5% N, 15% P_2O_5, 48% K_2O. The foliar application of *A. platensis* increased the yield in the first and second harvests compared to the NPK treatment, however, the yields were almost the same for *A. platensis* and NPK applications during the third harvest, and the yield was higher in NPK treated crops in the fourth harvest. The initial increase was explained by the high content of free amino acids in the *A. platensis* product, as well as the presence of growth-promoting substances directly absorbed by the leaves which takes place faster than the absorption of nutrients from the soil (Aly and Esawy, 2008). Therefore, it is demonstrated that algal biomass can be used directly onto crops via the leaves or roots to accelerate plant development or increase overall yield. Additionally, extracellular exudates from *Chlorella sorokiniana* in culture medium improved the total dry biomass (by 22% above ground and 51% below ground) and plant length of wheat plants (by 30%) compared to the control, highlighting the biostimulant potential of extracellular metabolites from microalgae (Kholssi et al., 2019).

Biostimulants can also affect root development which in turn affects the growth, health and nutritional composition of the whole plant because an increased number of lateral roots improves water and nutrient uptake (Garcia-Gonzalez and Sommerfeld, 2016). *Chlorella kessleri* extract was rich in phytohormones such as auxin and gibberellins (GAs) and upon application of aqueous extract to *Vicia faba*, it improved germination, seedling growth parameters, leaf area, pigment content and accumulation of sodium and potassium in roots and shoots,

compared to the controls (El-Naggar et al., 2005). *Scenedesmus quadricauda* and *Chlorella vulgaris* extracts have biostimulant effects on the expression of root traits and genes that are linked to nutrient acquisition in sugar beet, *Beta vulgaris* (Barone et al., 2018). *S. quadricauda* developed a higher number of root tips compared to plants treated with *C. vulgaris,* and seedlings treated with either algal species showed significant improvements in nutrient uptake due to increased total root length, fine root length and a number of root tips compared to controls. No significant differences were found between total root length, root surface area, and a number of root tips that were applied with one or two doses of microalgae, proving that minimal quantities have beneficial effects. When a biostimulant created from defatted *Nannochloris* sp., was tested on water-stressed and non-water stressed tomato plants, the latter obtained better development of root length, increased number of leaves and larger leaf area by 108%, 120% and 105% respectively compared to the controls, whereas the results for commercial macroalgae extracts showed 105%, 106% and 104% increases respectively. The water stressed plant's height and root length decreased by 20% and 25% respectively but the application of the *Nannochloris* sp. biostimulant alleviated the effects of water stress on root development and reduced the negative effects on plant height by 50% (Oancea et al., 2013). *Scenedesmus* sp. and *A. platensis* were foliar sprayed onto *Petunia x hybrid* at 10 g L^{-1} on days 0, 14, 28, 35 and 42. *Scenedesmus* accelerated root growth, leaf and shoot development, whereas *A. platensis* enhanced the root dry matter, number and dry weight of flowers and water content, however, it also reduced stem and petiole dry weight (Plaza et al., 2018). This demonstrates that biostimulant compounds differ depending on the algal species, so there is potential to combine microalgal species to improve all of the aforementioned traits.

Microalgae can also reduce the effects of salt stress on crops. Excess salt is toxic to plants and causes ion imbalances and osmotic stress (Zhu, 2001). The application of extracts from *Dunaliella salina* and *Phaeodactylum tricornutum* mitigated salt stress during germination of pepper plants whilst enhancing root growth and increasing germination rate (Guzmán-Murillo et al., 2013). In comparison to the control samples, the microalgae significantly reduced superoxide radical production and significantly increased the presence of antioxidant enzymes, implying that salt stress alters oxidative metabolism. Similarly, *Arthrospira maxima* and *Chlorella ellipsoida* extract enhanced the production of antioxidants and proteins in wheat grains, which improved wheat tolerance to salinity (Abd El-Baky et al., 2010). Further still, it has been concluded that extracellular products of *Scytonema hofmanni*, cyanobacteria, contain GAs-like plant growth hormones which counteract the effects of altered hormone homeostasis of salt-stressed rice seedlings (Rodríguez et al., 2006). Overall, salt stress can be alleviated through different mechanisms and the addition of microalgal biostimulants can aid these processes.

Biostimulants from microalgae also improve germination, flowering and fruit production of crops. However, it is important to note that the application type can have a significant effect on plant biostimulant activity. For example, cellular extracts and whole dry biomass of *Scenedesmus dimorphus* were applied to Roma tomato plants and the effects of application as a seed primer, foliar spray and biofertilizer were evaluated. When applied at over 0.75 g mL^{-1}, the seed primer caused germination to occur two days earlier and lateral root development was greater. Foliar application of the extract at 3.75 g mL^{-1} increased plant height, number of flowers, number of branches per plant and caused early fruit development. Applying dry algal biomass to the soil 22 days before transplantation enhanced plant growth, number of flowers and branches per tomato plant compared to a same-day application (Garcia-Gonzalez and Sommerfeld, 2016). Likewise, when *A. platensis* was applied at different foliar doses on aubergine plants, the low concentration (10 g L^{-1}) was identified as optimal as this increased fruit yield compared to control and higher concentrations (Dias et al., 2016).

2.2 Use of algal-bacteria and algae consortia to advance crop production and quality

Several studies have highlighted the potential of bacterial plant biostimulants. Examples of such plant growth-promoting rhizobacteria (PGPR) include non-pathogenic *Pseudomonas* and *Bacillus*, *Azotobacter*, *Serratia* and *Azospirillum* (Rouphael and Colla, 2020; Woo and Pepe, 2018). *Azospirillum brasilense* is recognised as plant growth-promoting bacteria (PGPB) and is known to promote the growth of many terrestrial plants and improve yields of numerous crop plants upon seed or root inoculation (Gonzalez and Bashan, 2000). Given that, it has been proposed that co-immobilization of microalgae and PGPB is an effective means of increasing microalgal populations within confined environments and later to improve the biostimulant potential of algal-bacterial consortia. For example, *Lupinus termis* seeds grown with cyanobacterial filtrates (*Anabaena*

flos-aquae and *Nostoc muscorum*) and bacterial suspensions (*Azotobacter chroococcum* and *Azospirillum brasilense*) significantly increased the average germination by 53.13%, 211.48%, 129.04%, and 104.18%, compared with untreated samples and those treated with hormones IAA, GA3 and cytokinins respectively (Tantawy and Atef, 2010). Furthermore, an algal-bacteria consortium mainly composed of PGPB (*Bacillus licheniformis, Bacillus megatherium, Azotobacter sp., Azospirillum sp.,* and *Herbaspirillum sp.*) and microalgae (*C. vulgaris*) was evaluated for its influence on the yield and nutritional parameters of leaf and romaine lettuce (Kopta et al., 2018). Application of such consortia at 14-day intervals led to 18.9% and 12.9% increases in weight for the romaine lettuce, and 16.5% and 22.7% increases in leaf lettuce in the spring and summer respectively. The carotenoid content of the treated romaine lettuces increased by 26.7% during the summer compared to controls showing that the algal-bacterial consortia reduced the negative effects caused by excess light and heat, allowing better development of light-dependent metabolites. In the summer, the total antioxidant capacity of romaine lettuce for algal-bacterial treatment was 2.5 times higher than the control (Kopta et al., 2018). It has also been noted that salt and temperature stress result in increased antioxidant activity, where elevated concentrations of the antioxidant enzyme guaiacol-specific peroxidase in French beans was reported (Babu and Devaraj, 2008). Despite this implying the plants were stressed due to high summer temperatures, the addition of the biostimulant consortia improved biomass compared to the control plants.

Consortia of algal strains without bacteria can also have a synergistic effect in improving plant yield (Renuka et al., 2018). It has been demonstrated that exopolysaccharide (EPS) production was highest with a co-culture of *Anabaena cylindrica* and *Nostoc* sp. compared to mono-cultures and this consortia application showed the highest plant yield in lettuce crops compared to mono-cultures implying that the EPS was beneficial (Xue et al., 2017). Generally, cyanobacteria aid in N-fixation, improve soil quality and biocontrol and Chlorophyta enhance growth and nutritional quality. This implies that a combination of the two strains or more, is likely to improve more agricultural aspects when compared with just one species, and it is possible to select specific combinations of synergistic traits, depending on the desired outcome (Renuka et al., 2018). For example, the application of a Cyanophyta (sp. of *Phormidium, Anabaena, Westiellopsis, Fischerella* and *Spirogyra*) and Chlorophyta (sp. of *Chlorella, Scenedesmus, Chlorococcum* and *Chroococcus*) consortia on wheat crops improved grain yield by up to 48% as well as improving the activity of microbes and the nutrients in the soil especially zinc, iron, copper and manganese which were also elevated in the wheat grains (Renuka et al., 2017). However, systematic selection of consortia partners is key to fully exploiting the feasibility of natural co-culture phenomenon (Padmaperuma et al., 2018). Consequently, further research into the effects of different combinations of algae should take place to confirm whether they should be cultivated together or whether the mutual benefits will still occur if cultured individually and then applied simultaneously.

3 Algal metabolites with biostimulant potential

3.1 Phytohormones

Phytohormones are naturally occurring small signalling molecules, produced in low concentrations that act as chemical messengers for regulation and stimulation of overall growth and development in terrestrial plants (Dmytryk and Chojnacka, 2018; Pan et al., 2019). The presence of phytohormones such as auxins, cytokinins, GAs and brassinosteroids (BRs) are well documented in both microalgae and macroalgae (Tarakhovskaya et al., 2007). Recent genomic investigations highlighted the biosynthetic and signalling processes of phytohormones in microalgae, and it was suggested that plant hormones originally evolved from microalgae between about 480 to 360 million years ago (Kenrick and Crane, 1997). Microalgae are capable of accumulating phytohormones in the cells and secreting them into the extracellular medium. The functional role of phytohormones in microalgae is elusive and still under investigation, however, recent literature indicated that phytohormones have similar regulatory roles in microalgae as those in higher plants (Lu and Xu, 2015). In this context, the application of microalgal phytohormones as biostimulants to plants or soils is gaining wide attention. Such application has promoted many essential physiological processes in plants such as cell division, growth, and differentiation, organogenesis, dormancy and seed germination, ageing, leaf pigments and the response to biotic and abiotic factors (Michalak et al., 2015). As biostimulants, the following major phytohormones are of commercial interest and found in algae: auxins, cytokinins, GAs, ethylene, abscisic acid, polyamines, BRs, lunularic acid (Niemann and Dörffling, 1980; Pryce, 1972), jasmonic acid, betaines,

rhodomorphin and salicylic acid (Oancea et al., 2013; Santner et al., 2009; Tarakhovskaya et al., 2007). Table 1 summarises key phytohormones, their biosynthesis, physiological actions in plants and key algae sources, whereas Table 2 and Table 3 summarise the highest reported concentration of phytohormones found in algae. A simultaneous determination of nine phytohormones in *Arthrospira* sp. and *Cladophora glomerata* (macroalgae) has been demonstrated (Górka and Wieczorek, 2017). Five of the nine were identified in higher concentrations in *C. glomerata* (indolacetic acid, phenylacetic acid, trans-zeatin, kinetin and 6-benzylaminopurine) and four of the nine were found in higher concentrations in *Arthrospira* sp. (isoentenyladenine, abscisic acid, indolebutyric acid and naphyleacetic acid).

Table 1

3.1.1 Auxins: Examples of auxins include indole-3-acetic acid (IAA), 4-chloroindole-3-acetic acid (ClIAA), indole-3-butyric acid (IBA) and 2-phenylacetic acid (PAA) (Górka et al., 2015). Auxins are reported to be found in brown algae (*Macrocystis, Laminaria*), green algae (*Klebsormidium nitens, Acutodesmus obliquus, C. vulgaris, Chlorella pyrenoidosa, S. quadricauda and charophyte, Cladophora, Enteromorpha*) and red algae (*Botryocladia*) (Liu et al., 2016; Ohtaka et al., 2017; Pan et al., 2019; Piotrowska-Niczyporuk et al., 2018; Tarakhovskaya et al., 2007) (Table 1). Sixteen marine algae were characterised for their IAA concentration, where the highest concentrations were reported in red alga *Polysiphonia urceolata* (110.2 ng g^{-1}) (Table 2) and green alga *Ulva pertusa* (81.4 ng g^{-1}), whereas brown algae had the lowest concentrations (Lijun, 2006). IAA is produced in cyanobacteria, *Nostoc* spp. (Sergeeva et al., 2002) and *Anabaena* sp. (Prasanna et al., 2008). Cyanobacteria and chlorophytes produce cytokinin-like compounds yet neither have high levels of auxin-like compounds, and cyanobacteria have additional potential due to nitrogen-fixing in the soil which creates a biofertilizer effect (Stirk et al., 2002; Win et al., 2018). When 24 algal strains were analysed, the higher intracellular auxin concentrations in faster-growing strains suggested that auxin is involved in cell growth and division in microalgae (Wendy A. Stirk et al., 2013). Auxins are also known for their use in agriculture due to herbicide and pesticide properties (Munira et al., 2018; Quareshy et al., 2018). Cyanobacteria excrete indole acetic acid, amino acids and other growth promoting compounds into their immediate environment, which can stimulate the growth of microbial populations in soil and thereby improve crop and soil quality due to interactions with bacteria (Karthikeyan et al., 2009). The extracellular cyanobacterial extract from *Aphanothece sp.*, rich in IAA, is reported to have similar regenerative activity as commercial phytohormones for use in *in-vitro* micropropagation of *Arachis hypogaea* and *Moringa oleifera* plants (Gayathri et al., 2015). Similar activity has been demonstrated with microalgal and cyanobacteria extracts on anther cultures of maize (Jäger et al., 2010). Likewise, cyanobacterial extracts containing IAA and cytokinins are reported to improve the plant growth parameters and resistance to biotic and abiotic stress factors (Hussain and Hasnain, 2011; Mazhar et al., 2013; Singh, 2014). A recent investigation demonstrated that the application of *Scenedesmus* sp. and *Arthrospira* sp. enhanced *Petunia x hybrida* root dry weight where *Scenedesmus* sp. was more effective due to higher concentrations of IAA (5965 ng g^{-1}) (Table 2) and abscisic acid (3718.25 ng g^{-1}) (Table 3) (Plaza et al., 2018). 2,4-dichlorophenoxyacetic acid is a widely used synthetic auxin in agricultural practices to mimic the actions of IAA, however, constant use leads to accumulation in the environment which can be detrimental to the treated plants (Górka et al., 2015).

3.1.2 Cytokinins: Algal cytokinins include zeatin, zeatin riboside, kinetin, isopentenyladenosine (IPA), 6-benzylaminopurine and topolin conjugates (Górka et al., 2015; Pan et al., 2019) (Table 2). Cytokinins-like plant biostimulant activity was initially reported in the early 1970s in macroalgae extracts (Williams et al., 1981). In the 1980s, 18 algal species extracts were found to possess similar biostimulant activity, with the highest concentration found in *Sargassum heterophyllum* during the onset of vegetative growth and gamete production (Mooney and van Staden, 1986; Mooney and Van Staden, 1984). Further research revealed the influence of seasonal variation, the age of the plants and harvesting times on the chemical composition of the macroalgae extracts. The highest concentrations of cytokinins and O-glucoside conjugates were reported in winter, whereas summer leads to accumulation of free bases. Also, glucoside conjugates were influenced by age, where the highest concentration of free bases and O-glucosides were detected in young tissues and old blades respectively (De Nys et al., 1990). Cytokinin profiles in 31 different macroalgal species were similar, all containing isoprenoid cytokinins, regardless of taxonomy, and the most prevalent cytokinins were *cis*-zeatins and isopentenyladenine derivatives (Stirk et al., 2003). In brown macroalgae, 18 cytokinins in an extract from

Ecklonia maxima and 19 cytokinins from *Macrocystis pyrifera* were detected with HPLC, where trans-zeatin-O-glucoside was the most common (Stirk et al., 2004). Increased storage time also caused increased cytokinin concentration. Seven auxin conjugates were detected in both species, and auxin concentrations were ten times higher than cytokinin concentrations. Indole-3-acetic acid was the main auxin in both of these macroalgae concentrates, however, total auxin concentration decreased with storage time. Due to the positive effects caused by low doses of macroalgae extracts on crops, a combination of plant growth regulators are likely to be the active ingredients. Foliar application of *Ascophyllum nodosum* extract at 10 µM, containing cytokinins and zeatin riboside, improved the heat tolerance of creeping bentgrass (Zhang et al., 2010). Furthermore, foliar application of aqueous macroalgae extract containing cytokinins was also reported for their antifungal and antibacterial properties against the tomato pathogens (Esserti et al., 2017).

Microalgal cytokinins play a crucial role in alleviating the effects of abiotic stresses. In *Nannochloropsis oceanica*, the sophisticated antagonistic regulatory role of cytokinins along with abscisic acid was demonstrated in alleviating the effects of nitrogen stress, where transcriptional upregulation and downregulation of abscisic acid and cytokinins biosynthetic pathways, respectively was reported (Lu et al., 2014). Besides, cytokinins were found to stimulate cell cycle progression whereas abscisic acid was found to suppress growth and enhance stress tolerance. In the case of plants, commercial cytokinins were reported to improve the yield of cotton seedlings by 10% in drought conditions (Burke, 2010). The exogenous application of *Nannochloris sp.* extracts containing cytokinins along with other components was reported to alleviate the water stress effects in tomato plants (Oancea et al., 2013). In terms of highest reported cytokinins concentration in microalgae, *Scenedesmus* sp. contains higher concentrations of zeatin riboside (6.82 ng g^{-1}) and isopentenyl adenine (45562 ng g^{-1}), whereas *Arthrospira* sp. contained a high concentration of trans-zeatin (191.37 ng g^{-1}) (Plaza et al., 2018) (Table 2).

Table 2

3.1.3 Gibberellins: More than 130 GAs have been characterised in plants, and they are classified into C19 (one carboxylic group at C-7 position) and C20 (two carboxylic groups at C-7 and C-19 position) GAs (He et al., 2020). Only a few forms are biologically active in flowering plants which include GA1, GA3, GA4 (~1000 fold higher activity than GA1) and GA7 (Eriksson et al., 2006). Similarities in protein sequences between algae and *A. thaliana* suggest that the biosynthetic pathways of GAs in algae match those in plants (Kiseleva et al., 2012). In concentrates of the macroalgae *A. nodosum*, gibberellin-like biostimulant activity was observed with very little effect. Moreover, the activity was found to be very unstable and disappeared after four months of storage (Stirk and van Staden, 2006). However, in *E. maxima*, 18 GAs were identified with the highest reported concentration (Table 2), seven of which were considered to be possessing biostimulant activity (Stirk et al., 2014). In cyanobacteria, extracellular extracts of *Scytonema hofmanni* containing gibberellin-like plant growth regulators were demonstrated to alleviate salt stress in rice seedlings (Rodríguez et al., 2006). In the case of microalgae, 18-20 GAs were detected from 24 microalgal strains that were analysed after four days of growth. The concentration detected was in the range of 342.7 pg mg^{-1} dry weight in *Raphidocelis subcapitata* to 4746.1 pg mg^{-1} dry weight in *Scotiellopsis terrestris*, however, in all strains the active GAs detected in the highest concentration was GA6. Slower growing strains of microalgae (such as *Scotiellopsis terrestris*) accumulate higher intracellular GAs and BRs than faster growing strains (such as *R. subcapitata* and *Coelastrum excentrica*) (W A Stirk et al., 2013) (Table 2). Recently, exogenous application of GA3 to microalgal cultures was investigated to boost the accumulation of commercial products, for example, for improving astaxanthin production in *H. pluvialis* (Gao et al., 2013) and protein, pigments and monosaccharides content and heavy metal remediation by *C. vulgaris* (Falkowska et al., 2011).

3.1.4 Brassinosteroids: In plants, BRs resemble the structure of ecdysteroids, an animal steroidal hormone (Bajguz and Hayat, 2009), whereas, in *C. vulgaris*, they have been reported structurally as hydroxylated derivatives of cholestane with some forms as conjugates with sugars or fatty acids (Górka et al., 2015). So far, around 69 BRs have been identified in plants, with brassinolide being the most potent (Ohnishi, 2018). BRs, brassinolide and castasterone are present in the stipes (25 pg mg^{-1} total) and fronds (13.8 pg mg^{-1} total) of *E. maxima* (Stirk et al., 2014). In terms of microalgae, they were detected in all 24 microalgal strains that were analysed in concentrations from 117.3 pg mg^{-1} DW in *R. subcapitata* to 977.8 pg mg^{-1} DW in *K. flaccidum* (Table 3). Brassinolide and castasterone were present in all 24 strains and generally, brassinolide existed in

higher concentrations (Stirk et al., 2013). Foliar application of BRs to tomato (Ogweno et al., 2008) and snap bean (El-Bassiony et al., 2012) plants can alleviate the adverse effects of heat stress and improve overall plant growth by increasing the carboxylation efficiency and antioxidant activity in leaves. The cross-talk and synergistic effects between BRs and other phytohormones such as auxins, abscisic acid, GAs, ethylene and salicylic acid are evident (Bajguz and Hayat, 2009). For example, in the case of *A. thaliana*, co-application of BR with GA or auxin resulted in a synergistic increase in hypocotyl elongation. On the other hand, co-application of BR with abscisic acid resulted in cell elongation in *A. thaliana* mutants and increased drought resistance in *Sorghum vulgare* (Bulgari et al., 2019).

Table 3

3.1.5 Ethylene: For various agricultural applications, ethylene is widely used and extracted from *Chlamydomonas* sp., *Chlorella* sp., *Scenedesmus obliquus*, blue-green algae, marine red algae and coral reef algae (Lu and Xu, 2015; Pan et al., 2019) (Table 1). A transcriptomic investigation identified a large set of ethylene-regulated genes in green alga *Spirogyra pratensis*, where around 10 ethylene-responsive transcription factor homologs regulated by the ethylene application and ethylene was shown to regulate photosynthesis, chlorophyll biosynthesis, cell remodelling and responses to abiotic stresses (Van de Poel et al., 2016). In case of microalgae, *Scenedesmus* sp. and *Arthrospira* sp. were reported to contain the highest concentration of ethylene, 341 ng g^{-1} and 546 ng g^{-1} respectively (Plaza et al., 2018) (Table 3).

3.1.6 Abscisic acid: Abscisic acid is 15-C sesquiterpenoid and in roots is linked to high levels of ethylene which suppresses root growth and contrastingly, high abscisic acid levels suppress ethylene synthesis, which reduces auxin transport and biosynthesis in the root tip and promotes root growth (McAdam et al., 2016). Leaf hydration is the main signal for regulating plant responses to moisture and the concentration of foliar abscisic acid can be used as a signal for root growth (McAdam et al., 2016).

In macroalgae, abscisic acid has been detected in 64 species (Hirsch et al., 1989) and isolated from *A. nodosum* at concentrations of 0.03-0.15 µg g^{-1} DW (Boyer and Dougherty, 1988) (Table 3), seven macroalgal species (ten strains in total) from Brazil (Yokoya et al., 2010), *Ulva saciata* and *Dictyota humifusa* (Stirk et al., 2009), Brazilian red algae (Yokoya et al., 2010) and *Laminaria japonica* (Nimura and Mizuta, 2002). In the case of microalgae, *C. vulgaris*, *C. sorokiniana*, *N. oceanica*, *D. salina*, *Chlamydomonas reinhardtii*, *Haematococcus pluvialis* and *S. quadricauda* are well-known species for their higher abscisic acid content (Lu et al., 2014; Pan et al., 2019; Yoshida et al., 2003). *Scenedesmus* has a higher concentration of cytokinins (isopentenyl adenine), GA (GA1), auxins (indoleacetic acid), salicylic acid, and abscisic acid compared with *Arthrospira* (Table 2 and 3) and therefore, foliar application of *Scenedesmus* to *Petunia x hybrida* plants resulted in an increased number of flowers, shoots, and leaves (Plaza et al., 2018). Abscisic acid is involved in alleviating the effects of salt stress in cyanobacteria (Maršálek et al., 1992), and nutrient stress in *D. salina* (Lu et al., 2014).

3.1.7 Jasmonic and salicylic acid: Jasmonic and salicylic acid play a central role in the regulation of plant defence responses and are present in nearly all algae that have been studied (Arnau and Richard, 2016). The delicate trade-offs between the plant development and activation of defence against pathogenic microbes and herbivorous insects involve cross-talks between other phytohormones and salicylic and jasmonic acid signalling pathways which are inter-dependent and have a complex network. Auxins, cytokinins and GAs which are involved in regulating plant growth are the strongest antagonists of jasmonic acid and salicylic acid signalling output. Abscisic acid and ethylene act synergistically with the jasmonic acid signalling output, however, antagonize salicylic acid responses. Jasmonic acid regulates defence responses against herbivorous insects and necrotrophic pathogens, while salicylic acid regulates defence responses against biotrophic pathogens (Caarls et al., 2015; Pieterse et al., 2014; Santner et al., 2009). Jasmonic acid has been associated with salt stress defence mechanisms in plants such as sweet potatoes (Zhang et al., 2017). Linoleic acid is a precursor of jasmonic acid which decreases in stressed plants because jasmonic acid initiates mechanisms of plant defences to salt stress, therefore, linolenic acid must be utilised to upregulate jasmonic acid (Pedranzani et al., 2003).

Jasmonic acid is found in nearly all algal species and in *Chlorella* and *Lithothamnion*, some components of the octadecanoid pathway of jasmonic acid biosynthesis from linolenic acid were detected (Tarakhovskaya et al., 2007). This implies that the addition of these species to crops may alleviate salt stress. The highest

concentration of jasmonic acid and salicylic acid were reported in *Scenedesmus* sp., 75.13 ng g^{-1} and 156714 ng g^{-1} respectively (Plaza et al., 2018) (Table 3). Overall, phytohormones have individual roles in plants yet the metabolic pathways overlap and the interaction between different hormones allow growth and development of the whole plant.

3.1.8 Polyamines and betaines: Polyamines and betaines are nitrogen-containing compounds found broadly across taxonomic groups from bacteria to mammals (du Jardin, 2012). The major forms of polyamines are putrescine, spermine, and spermidine (Kusano et al., 2008). Enzymatic hydrolysis of *A. platensis* at 2ml L^{-1} for four hours resulted in a 34% increase in spermine content in the hydrolysate compared to the non-hydrolysed extract. The foliar application of such hydrolysed *A. platensis* extract promoted the growth of lettuce seedlings and increased the spermine content in the leaves by 64% (Mógor et al., 2018b). Polyamines accumulate in stressed plants so by applying them as a biostimulant, plants will be more resistant to stress factors (Papenfus et al., 2013).

Betaines acts as an osmolyte and regulate defence mechanisms against abiotic stresses such as salinity, frost and drought (Khan et al., 2009). Betaines are present in macroalgae to allow resistance to salt stress, as they inhibit the effect of high salt concentrations and lack of water due to tides. They are also known to prevent chlorophyll degradation and allow longer photosynthetic retention in salt-stressed plants (Yuvaraj and Gayathri, 2017). Betaines such as glycine betaine, γ-aminobutyric acid betaine, δ-aminovaleric acid betaine and laminine are reported in *A. nodosum*, *Laminaria* spp. and *Fucus serratus* (Blunden et al., 2010; MacKinnon et al., 2010).

3.2 Protein hydrolysates and amino acids

Both protein hydrolysates (PHs) and amino acids represent a major category within plant biostimulants and are extensively used in sustainable agricultural practices (Bulgari et al., 2019). Protein hydrolysates are a mixture of mainly short peptides (polypeptides, oligopeptides) and free amino acids, which may also contain a small proportion of fats, polysaccharides, phytohormones and macro and micronutrient elements (Calvo et al., 2014; Colla et al., 2017). Currently, most commercial products containing PHs such as Siapton, Alfalfa, Macro-Sorb Foliar and Aminoplant are produced in Spain, Italy, USA, India and China from animal wastes or plant biomass by chemical (acid/alkali), thermal or enzymatic hydrolysis (Calvo et al., 2014). The total concentration of peptides and key free amino acids (such as alanine, arginine, glycine, proline, glutamate, glutamine, valine and leucine) varies from 1 to 85% (*w/w*) and 2-18% (*w/w*) respectively (Calvo et al., 2014). Table 4 summarise the highest reported concentration range of total proteins and key amino acids derived from algae. PHs are known to have direct effects in modulating carbon and nitrogen metabolism in plants, thereby regulating the nutrient uptake and key enzymes (citrate synthase, isocitrate dehydrogenase and malate dehydrogenase) in a tricarboxylic acid cycle (Colla et al., 2017). The bioactive peptides exert auxin and gibberellin-like activity in plants, enhancing overall growth and crop yield (Colla et al., 2017; Ertani et al., 2013), whereas, proline and glycine betaine are known to contribute to micronutrients mobility and acquisition along with mitigation of environmental stresses via chelating effects (protects the plant from heavy metals) and antioxidant activity (du Jardin, 2015). In contrast, the indirect effects of PHs include enhancing nutrient availability and nutrient uptake in plants via promoting soil respiration and growth of PGPB in the plant microbiome (Bulgari et al., 2019; du Jardin, 2015; Paul et al., 2019).

Table 4

Application of macroalgal or microalgal extracts containing individual amino acids to plants is known to increase the synthesis of proteins, pigments and key phytohormones responsible for plant growth (Guedes et al., 2018). For example, tryptophan is a precursor of plant hormones auxin, salicylic acid and aromatic secondary compounds which have multiple biological functions in plants, whereas arginine is a precursor to polyamines (Bulgari et al., 2019), which are involved in many important biological processes as described in section 2.1.8. The mode of action and key metabolic pathways involved in biostimulant activity of PHs and amino acids are yet to be explored, however, it is important to note that many factors influence the biostimulant activity of PHs such as source (plant or animal origin), extraction/hydrolysis technique, composition and solubility, application type, timing, concentration applied, cultivars, leaf permeability and phenological stages (Colla et al., 2017). For example, severe growth depression of tomato plants was observed with root and foliar application of animal-

483 derived amino acids, whereas plant-derived amino acids stimulated the growth, chlorophyll and Fe content
484 (Cerdán et al., 2013). This might be due to the role of glutamic acid in nitrogen metabolism and chlorophyll
485 biosynthesis. Several reports also suggested that amino acids such as glutamate, phenylalanine, cysteine,
486 histidine, proline, glycine and glycine betaine are important for metabolic signalling and mitigating the effects
487 of environmental stresses such as heavy metals, nutrients, oxidative, heat, cold, drought and salinity (Korkmaz
488 et al., 2010; Liang et al., 2013; Paul et al., 2019; Popko et al., 2016; Sharma and Dietz, 2009, 2006; Teixeira et
489 al., 2017).

490 The application of hydrolysate containing intracellular amino acids derived from *Cylindrospermum*
491 *muscicola* to rice plants promoted root growth, however, a later investigation involving the application of 14
492 individual amino acids to rice seedlings suggested cystine, tyrosine and phenylalanine are the main bioactive
493 amino acids responsible for the plant growth (Venkataraman and Neelakantan, 1967). Certain microalgal strains
494 are reported to have over 40% dry weight amino acid content which includes *A. platensis* 46.8%, *A.*
495 *maxima* 44.9%, *Chlorella* sp. 44.3% and *C. saccharophila* 42.4% (Hempel et al., 2012), making them ideal
496 candidates for the development of biostimulants products. The foliar application of lyophilised *A. platensis*
497 suspension to red beet promoted the chlorophyll, protein, sugars, and free amino acid contents along with
498 improved hypocotyl growth (Mógor et al., 2018a). The positive effects could be due to the free amino acids in
499 the biomass, as *A. platensis* is rich in essential amino acids, such as aspartic acid, glutamic acid, arginine,
500 threonine, alanine, isoleucine, and leucine. Similarly, foliar application of protein hydrolysate (obtained via
501 enzymatic hydrolysis) of *Arthrospira* and *Scenedesmus* biomass (at 10 g L^{-1}) to *Petunia x hybrida* plant resulted
502 in increased number of flowers, the flower fresh and dry matter per plant and the root dry weight (Plaza et al.,
503 2018).

504 ## 3.3 Humic substances

505 Humic substances (HSs) are commonly mentioned in lists describing types of biostimulants, however,
506 their source from algae has not been widely researched. HSs, as with any other biostimulant, behave differently
507 depending on environmental conditions, timing and rate of application, and which plant species you are applying
508 it to (Rose et al., 2014). HSs are natural constituents of soil organic matter (~60%) and are formed by the
509 decomposition of plants, animals and microbial residues or from the metabolic activity of soil microbes using
510 these substrates (du Jardin, 2015). Based on their molecular weights and solubility, HSs are categorised into
511 humic acids, fulvic acids and humins (du Jardin, 2015). The majority of HSs currently used in agriculture are
512 derived from non-renewable resources such as coal, peat or mineral deposits, therefore, sustainable alternatives
513 need to be developed such as from composts and vermicomposts (Canellas et al., 2015; du Jardin, 2015). HSs
514 are considered as biostimulants and have reportedly improved growth aspects in over 16 plant species (Calvo et
515 al., 2014). However, the molecular mechanism underlying the biostimulant activity of HSs is unclear and is
516 believed to be very complex. Results from the application of HSs on plants show inconsistent yet overall positive
517 results on plant growth with the majority of biostimulant effects being related to the improvement of root
518 nutrition via different mechanisms (du Jardin, 2015). Therefore, an understanding of the interplay between the
519 HSs, organic matter in the soil, microbes and plant roots is crucial to fully explore the biostimulant potential of
520 HSs (du Jardin, 2015).

521 Evidence suggests the presence of humic acid in the algal biomass, for example, humic acid (10 ± 1%
522 of the dry weight) in the brown filamentous algae *Pilayella littoralis* (Ghabbour et al., 1994). In terms of
523 microalgae, the acidification of *C. reinhardtii* resulted in precipitation of a brown solid with lack of solubility
524 in organic solvents, where it was concluded that it was a humic acid, although there are many types with complex
525 structures and this was not verified (Heilmann et al., 2011). In another report, an NMR technique demonstrated
526 that polysaccharide and peptide-rich microalgae consisting mainly of *Chlamydomonas intermedia* from Pony
527 Lake, Antarctica, were humified to fulvic acid in the lake (Mao et al., 2007). The potential of microalgae as a
528 HS producer needs to be investigated in further detail to assess whether this has a value on its own or as part of
529 a biorefinery approach.

530 Agricultural by-products, can undergo controlled breakdown and oxidation by chemical processes, that
531 create humic-like substances which could be used as substitutes for natural HSs (Eyheraguibel et al., 2008). The
532 liquid fraction of digestate from anaerobic digestion plants has poor biodegradability due to the presence of

humic acid-like and fulvic acid-like substances making aerobic treatment inefficient (Akhiar et al., 2017). The use of microalgae to bioremediate the liquid fraction of digestate is a feasible new technology that has already been demonstrated (Fernandes et al., 2020). Moreover, it has been demonstrated that fulvic acid can adsorb to the surface of *Scenedesmus subspicatus* at pH 4 and 5 which in turn increases the carbon uptake (Knauer and Buffle, 2001). Theoretically, HSs in the digestate may be able to bind to the algal cells and then later have a biostimulant effect when applied to crops. In contrast, humic substances extracted from agro-industrial waste were evaluated for their biostimulant effect on *C. vulgaris* and *S. quadricauda*, where a significant increase in biomass, lipids, chlorophyll and carbohydrates content was reported (Puglisi et al., 2018). Recently, the combined effects of *S. subspicatus* biomass and a humic acid mixture was compared against their individual biostimulant activity on onion bulbs. Plant growth occurred during early stages when onion seedlings were immersed in a solution containing a mixture of 0.30 g L^{-1} *S. subspicatus* and 0.30 g L^{-1} humic acid. Improved bulb calibre with a significant increase in bulb sugar and protein content has also been reported (Gemin et al., 2019).

3.4 Polysaccharides

Algal polysaccharides are claimed to have biological and therapeutic benefits in food, pharmaceutical and nutraceutical applications (Llewellyn et al., 2019). Polysaccharides are known to be involved in numerous plant metabolic pathways, and therefore can act as an excellent source of potential plant biostimulants for overall crop improvements and protection against biotic and abiotic stresses (Rachidi et al., 2020). The key polysaccharides found in macroalgae are ulvans, galactans (agarans and carrageenans), fucoidans, laminarans, alginates and oligoalginates. However, the structural and biochemical composition of macroalgal polysaccharides varies greatly. Ulvans are more dominant in green macroalgae (around 8 to 29% DW), galactans such as agarans and carrageenans (κ, ι, and λ forms) in red macroalgae (Rhodophyta: species of *Eucheuma, Chondracanthus, Gigartina, Hypnea, Kappaphycus*) and alginates, fucoidans, and laminarans in brown macroalgae (Phaeophyceae: species of *Ascophyllum, Fucus, Pelvetia, Laminaria, Lessonia, Sargassum*) (Dmytryk and Chojnacka, 2018; Górka et al., 2015) (Table 5). The main constituents of macroalgal polysaccharides are hexose, pentose, uronic acid, rhamnose, fucose, fructose and methyl sugars (Pan et al., 2019; Raposo et al., 2013). The derived oligosaccharides of ulvans, alginates, fucans, laminarin and carrageenans can activate salicylic acid, jasmonic acid or ethylene signalling pathways in plants causing increased expression of pathogenesis-related proteins. These proteins have antifungal and antibacterial effects and increased expression of defence enzymes that synthesise compounds that have antimicrobial properties (Vera et al., 2011). Carrageenans mediate the plant growth and plant defence responses via modulation of jasmonate, salicylate and ethylene signalling pathways and other metabolic processes (photosynthesis, nitrogen and sulfur assimilation) (Shukla et al., 2016).

Table 5

The most common microalgal polysaccharides are heteropolymers of galactose, xylose, mannose, arabinose and glucose in different proportions linked by glycosidic bonds, where the only exception is a homopolymer of galactose (in *Gyrodinium impudicum*) and B-(1,3)-glucan (in *C. vulgaris*). The biostimulant activity of such microalgal polysaccharides is largely determined by proportional differences in constituent neutral sugars and by other factors such as degree of sulfation, uronic acid content and molecular weight (Chanda et al., 2019; Farid et al., 2019). In the case of microalgae, foliar application of total polysaccharide extract from *A. platensis* at 3 g L^{-1} (*w/v*) to tomato and pepper plants resulted in a significant increase in overall plant growth (Elarroussi et al., 2016). Plant size increased by 20% and 30%, leaf number increased by 50% and 33%, leaf area size increased by 100% and 57%, root weight by 230% and 67%, size of nodes by 57% and 33%, number of nodes by 100% and 50% for tomato and pepper plants respectively and shoot dry weight was increased by 140% in both plant species. Further studies with the exopolysaccharide extract from *D. salina*, containing sulphated moiety, carbohydrates and uronic acids, have been shown to alleviate salt stress in tomato plants preventing a decrease in the length and dry weight of shoots and roots, the decrease of potassium and therefore potassium:sodium ratio caused by salt stress. *D. salina* exopolysaccharides also prevented an increase in proline, phenolic compounds, Na^+, and antioxidant enzyme activities that occur in salt-stressed tomato plants (Arroussi et al., 2018). Such multi-functional positive effects on crops highlight the biostimulant potential of microalgal polysaccharides. The potential of microalgal polysaccharides in mitigating ROS toxicity in plants

has also been demonstrated, where polysaccharides were extracted from *C. vulgaris, C. sorokiniana, C. reinhardtii* and *D. salina* and injected into tomato plants for assessment of biostimulant activity (Farid et al., 2019). Extracts from *C. vulgaris* and *C. sorokiniana* exhibited a significant increase in β-1,3-glucanase activity after 48 hours, whereas *C. sorokiniana* extract had a significant stimulatory effect on phenylalanine activity with a 188.73% increase compared to the control. *D. salina, C. sorokiniana*, and *C. reinhardtii* extracts also increased PUFA content in the tomato plants by 50.37%, 34.46%, and 33.37% respectively. *C. reinhardtii* polysaccharides enhanced stearic acid, palmitic acid, and very long-chain fatty acid content, the optimal values of which increased by 45.50%, 32.83%, and 60.60% respectively under treatment compared with the control. *C. vulgaris* and *C. reinhardtii* polysaccharides also exhibited higher ascorbate peroxidase and peroxidase antioxidant activities respectively (Farid et al., 2019). Recently, the application of 1 mg mL^{-1} polysaccharide extract from *A. platensis*, *D. salina* and *Porphyridium* sp. was evaluated for their effects on a shoot and root length, dry weights and node numbers in tomato plants. A significant increase in all the parameters compared to control along with an increase in total carotenoid, chlorophyll and protein content and activity of nitrate reductase and NAD glutamate dehydrogenase in plant leaves were reported. Moreover, metabolome analysis showed an increase in the sterol precursors suggesting enhancement of sterol biosynthesis in the treated plants (Rachidi et al., 2020).

3.5 Antioxidants

Algae contain a range of compounds that can be classified as antioxidants including vitamins C and E, carotenoids, chlorophylls and phenolics (Shebis et al., 2013). Industrially cultivated samples *of Botryococcus braunii, Neochloris oleoabundans, Isochrysis* sp., *H. pluvialis, C. vulgaris, P. tricornutum, Turbinaria ornate, Gayralia oxysperma, Chaetomorpha antennina, Sargassum vulgare, Undaria pinnatifida, Himanthalia elongate* and *Chondrus crispus* have all been shown to have high antioxidant capacities (Shebis et al., 2013). As such there are opportunities to use antioxidants in microalgae to improve the growth of plants (Sakr et al., 2017).

Phenolic compounds are considered one of the most important classes of natural antioxidants. Several algal species are known to produce/release phenolic compounds as a part of a stress and defence response. Phenolic compounds are classified into phenols and polyphenols. Phenolic compounds obtained from brown algae include phenolic acids, flavonoids and phenylpropanoids (Pan et al., 2019). Overall, they are known to act as antioxidants, antimicrobials, antivirals and radioprotectives, and the application of such algal phenolics to plants is expected to have these effects (Dmytryk and Chojnacka, 2018). Polyphenols in plants are usually derived from gallic and allagic acid, whereas in algae they are derived from polymerized phloroglucinol units (Górka et al., 2015). Algal phlorotannins are an important group of polyphenolic compounds usually derived from the acetate-malonate pathway and are not found in plants. They are further subclassified into fuhalols, phlorethols, fucols, fucophloroethols and eckols. The highest concentration of phlorotannins (minimum 10% DW) was reported in brown macroalgae including *Ascophyllum* sp., *Ecklonia* sp., *Fucus* sp., *Sargassum fusiforme*, *Ishige okamurae*, *Saccharina japonica*, *Sargassum thunbergii*, and *Undaria pinnatifida* (Craigie, 2011; Dmytryk and Chojnacka, 2018; Górka et al., 2015). Eckol and phloroglucinol, isolated from *E. maxima* are reported to have a biostimulant effect on overall growth and physiological response in *Eucomis autumnalis* with increased concentrations of the flavonoid kaempferol and ferulic acid and a 1.5 fold increase in bulb size, compared to that of a commercial biostimulant, Kelpak (Aremu et al., 2015). Likewise, foliar application of eckol extracted from *E. maxima* is reported to improve the overall growth, myrosinase activity (aphid resistance capacity) and concentration of photosynthetic pigments and phytochemicals in commercially cultivated cabbage (Rengasamy et al., 2016). Macroalgae extracts containing micronutrients such as Zn and Mn enhanced cold tolerance in maize plants due to increased superoxide dismutase activity in root and leaf tissue. The biostimulant activity was attributed to the presence of micronutrients, as antioxidative stress defence mechanisms depend on micronutrients, such as Zn and Mn, as enzymatic co-factors (Bradáčová et al., 2016).

Secondary metabolites often act as antioxidants; blocking oxidative reactions induced by stresses and enhancing the antioxidant potential of vegetables, flowers and fruits (Bulgari et al., 2015). The antioxidant enzymes in salt-stressed pepper plants, *Capsicum annuum*, increased significantly when treated with extracts from *D. salina* and *P. tricornutum* (Guzmán-Murillo et al., 2013). Several other antioxidant compounds such as mycosporine-like amino acids, bromophenols, coumarins, vanillic acid, nitric acid, glutathione, ascorbic acid,

proline and abscisic acid are also reported in algae (Bulgari et al., 2019; Pan et al., 2019). The antioxidant activity upon exogenous application of glutathione, proline, abscisic acid, nitric acid and ascorbic acid has already been described in mung beans and chickpeas (Kaushal et al., 2011; Kumar et al., 2012, 2011; Nahar et al., 2015; Yang et al., 2006). Phenolic compounds produced by both *Chlorella* and *Arthrospira* include phloroglucinol, p-Coumaric acid, ferulic acid, and apigenin (Andrade et al., 2018), whereas fucoxanthin and gallic acid were reported to be the key components responsible for antioxidant activity in five microalgal and one macroalga species, where the highest antioxidant activity was found in *Chaetoceros calcitrans* and *Isochrysis galbana* (Foo et al., 2017). Algae are well known for the production of carotenoids such as α and β-carotene, lutein, lycopene, β-cryptoxanthin and zeaxanthin which can be used in agricultural practices as antioxidants (enhances provitamin A levels), fertilisers (for remediation of petroleum contaminated soil) and pesticides (Pan et al., 2019). *D. salina* is the most suitable microalga for the production of β-carotene, as it can produce up to 14% DW (Metting, 1996) (Table 5). Application of such algal antioxidant compounds to plants are likely to have a positive effect on the crops or encourage antioxidant activity within the plants, however, this remains largely unexplored. This might be due to the bottlenecks associated with the overall cost of production and the low current market value for agricultural applications.

3.6 Allelochemicals

Allelochemicals are defined as secondary metabolites produced from plants, animals or microorganisms that have ecological functions to suppress stressors (Gross, 2009). Allelochemical interactions between plants can be exploited to recover soils by using crop rotations, intercropping, cover crops and mulching but should also be considered for their biostimulant properties (du Jardin, 2015). Limited research has taken place thus far to identify the biostimulant properties from allelochemicals produced from algae and cyanobacteria, however, a diverse range of cyanotoxins are produced by cyanobacteria which have potential uses as allelochemicals that can inhibit microbes, insects or weeds, therefore, allowing better quality target crops (Rastogi and Sinha, 2009).

Cyanobacteria can produce toxic allelochemicals such as cyclic depsipeptides, cyclic peptides and volatile organic compounds that deter feeding by zooplankton, nematodes and snails. Microcystins and other hepatotoxic cyclic peptides produced by the cyanobacteria *Microcystis*, *Planktothrix*, *Anabaena*, and *Nodularia* inhibit protein phosphatases and can be fatal to mammals. It is reported to be unlikely that microcystins act as allelopathic compounds against photoautotrophs due to the low concentrations produced (Babica et al., 2006). Cyclic depsipeptides affect digestive enzymes and act as trypsin inhibitors in zooplankton such as *Daphnia magna*. Oscillapeptins are also protease inhibitors, found in *Planktothrix rubescence* and *Oscillatoria agardhii*. *Anabaena flosaquae* produces anatoxin-a, an alkaloid neurotoxin which affects growth and performance of zooplankton (Gross, 2009). *Scytonema hoffmanii* produces cyanobacterin an allelophatically active compound which targets algae and higher plants, negatively affecting growth and photosynthesis (Gleason and Case, 1986). Cyanobacterin inhibits the growth of angiosperms, including the aquatic, *Lemna*, and terrestrial species such as corn and peas. It was concluded that cyanobacterin inhibits oxygen-evolving photosynthetic electron transport in all plants and most probably in photosystem II (Gleason and Case, 1986). Pentacyclic calothrixins obtained from a *Calothrix* sp. cyanobacteria may inhibit RNA polymerase and DNA synthesis and hence act as allelopathic compounds and exert their growth-inhibitory effects at nanomolar concentrations (Rickards et al., 1999). The study investigated the malaria parasite and cancer cells, however, there is potential to investigate crop parasites. *Lyngbya majuscule* produces majusculamide-C, a microfilament depolymerising agent which has shown fungicidal activity and could, therefore, be used in the treatment of resistant fungal-induced diseases of plants and crops (Chakdar et al., 2012). *C. vulgaris* has been found to have phytoprotective effects on grape seedlings infected with *Xiphinema index* nematodes when applied at 1g $100ml^{-1}$, though the mechanism through which this occurred was not determined (Bileva, 2013). Therefore, there is potential for commercial exploitation of the above compounds for biopesticides (Rastogi and Sinha, 2009) but further research should take place to identify whether the application of these compounds can be species-specific to act as pesticides or herbicides. Commercial development and application of these compounds as biopesticides is predicted to be more environmentally beneficial in comparison to synthetic alternatives, however, further research *in vivo* is necessary as current research is mainly limited to *in vitro*.

3.7 Vitamins, terpenoids and free fatty acids:

Many reports mention that vitamins from microalgae may have a biostimulatory effect on crops however, there is limited evidence to support this (Mahapatra et al., 2018; Shaaban, 2001; Whitton, 2000). *Cylindrospermum muscicol*, cyanobacteria, can produce 1.2 to 1.5 µg g^{-1} vitamin B12 (Venkataraman and Neelakantan, 1967) (Table 5). When compared to 12 known growth factors *Cylindrospermum muscicola* biomass caused increased root growth of rice plants at a comparable rate to pure vitamin B12 implying that this vitamin is causing or contributing to the biostimulatory effect caused by the algae (Venkataraman and Neelakantan, 1967). Marine cyanobacteria produce vitamins that have commercial potential such as vitamin E and the B vitamins: biotin, pantothenic acid and nicotinic acid (Table 5). *Arthrospira* can produce vitamin A and is the richest known source of vitamin B12 (Chakdar et al., 2012) and therefore there is potential value in extracting B12 for biostimulant applications. More specific research must take place to identify the beneficiary effect of vitamins or the synergistic effect of vitamins with phytohormones, amino acids or polysaccharides from microalgae on crops.

Other algal metabolites with biostimulant potential that need to be explored include terpenoids and free fatty acids. Terpenoids have several recognised applications in agriculture such as antioxidants, antimicrobials, biopesticides, ecosystem intensification, post-harvest disease management, plant hormone stimulation and allelopathy, whereas free fatty acids boost overall plant growth via their antibacterial and antioxidant properties (Pan et al., 2019).

4. Extraction of specific biostimulatory compounds

Algal biomass can be used directly without extraction of specific compounds, where drying of the biomass is not required, and it can be concentrated to create fixed sludge biomass (Acién et al., 2016). For example, *Chlorella* sp. was grown on wastewater and wet biomass was applied directly to the soil. This caused the formation of microalgal soil biofilm, which enhanced plant-microbe interactions and resulted in improved biostimulatory and biofertilizer effects compared to controls (Castro et al., 2017; Marks et al., 2019). However, the product becomes more valuable if specific components are selectively extracted, concentrated and purified and the biomass used can be either wet or dry. Desirable products can be intracellular or extracellular. Extracellular products can be obtained by centrifugation or membrane filtration, however, extraction of intracellular products proves more difficult. To obtain intracellular products, cell disruption is essential to permeabilise the cell wall (where it exists) and/or cell membrane, and to allow for the extraction of intracellular metabolites with the aid of an extracting agent and later stabilise them for subsequent commercial applications (Kapoore, 2014). This can be achieved through methods which are broadly categorised as mechanical (using solid/liquid shear), chemical (solvents, acid/alkali, ionic liquids, chelating agents, detergents, osmosis, nanoparticles, supercritical fluids), thermal/thermo-chemical (autoclave, steam, hydrothermal liquefaction, freeze-drying), electromagnetic (microwaves, ultrasound), biological (antibiotics, enzymes, phage) and current (pulsed electric field) (Kapoore et al., 2018).

An ideal extraction method for microalgal biostimulants should: cover a broad range of bioactives or be selective as per requirement, be environmentally friendly and easily scalable, not be too harsh and should extract the bioactives without affecting their bioactivity, have high extraction yield, have minimal waste and have low cost, labour, energy, toxic solvents and time input. However, at present such an ideal extraction method does not exist, hence it is essential to develop existing methods to improve extraction efficiency and reproducibility for a given case (Kapoore, 2014). Interestingly, the chemical composition and biological activity of extracts can be considerably different from the same biomass if different methods and chemicals are used for disruption (Battacharyya et al., 2015). Therefore, when deciding on the extraction method it is important to consider other factors such as metabolite classes of interest, algal species under investigation, the balance between extraction yield and preservation of bioactives, phenological characteristics of algal species in terms of seasonal variation, the application type, target crop and desired physiological effects (EL Boukhari et al., 2020; Stirk et al., 2020). Algal extracts can be complex mixtures of various compounds and separation of specific compounds can vary depending on the level of cell disruption that has occurred.

Extraction is the most crucial step in the algal biostimulant industry which gives manufacturers a competitive advantage in capturing the market. Most of these methods are based on soft techniques such as low pressure and temperature to preserve the bioactivity of the molecules and are often subject to professional secrecy (EL Boukhari et al., 2020). Traditional mechanical and chemical extraction techniques are labour intensive, utilise high quantities of toxic organic solvents, can expose bioactives to excessive light, heat and oxygen, and can result in changes in stereochemistry (Kapoore et al., 2018). For instance, chemical methods significantly reduce the quality of the final products as phytohormones and L-amino acids can be degraded (Figueroa et al., 2018). Novel extraction techniques that have been tested for the production of macroalgae based biostimulants include ultrasound, enzymes, supercritical fluid, microwaves, pressurized liquids, water-based extraction, acid/alkali hydrolysis, and cell burst technology (Battacharyya et al., 2015; EL Boukhari et al., 2020; Shukla et al., 2019). In all cases, the bioactivity and composition of the extract were inconsistent and was mainly dependent on the extraction method employed (Shukla et al., 2019).

In terms of green extraction techniques, advantages and disadvantages of current disruption and extraction techniques have been recently reviewed and summarised in tabular form (Kapoore et al., 2018). Supercritical extracts of macroalgae have been reported to enhance the overall growth and development in wheat (Michalak et al., 2016). Likewise, under controlled lab conditions, supercritical CO_2 extract from *A. platensis* had a greater stimulatory effect on cucumber fruits than the commercial stimulants Asahi SL and Forthial, whereas, in field conditions, the extract enhanced overall growth in wheat plants at the lowest dose (Dmytryk et al., 2016). Pressurized liquid extraction (PLE) is another green technique that utilises high pressure and temperature, however, both supercritical fluid extraction and PLE are not currently suitable for scale-up due to the high energy input required for maintenance of high temperature and pressure. Microwave-assisted extraction (MAE) is an environmentally friendly option which can extract biochemical components selectively and as part of a biorefinery, with a lower solvent requirement, reduced working times and which results in higher yields and purity of products (Kapoore et al., 2018). So far MAE has been used for extraction of sugars, phenolic compounds, fucoidans and sodium alginates from macroalgae (Yuan et al., 2018b, 2018a; Yuan and Macquarrie, 2015) and for extraction of fatty acids, proteins, carbohydrates, vitamins, carotenoids and chlorophylls from microalgae (Ansari et al., 2017a; Esquivel-Hernández et al., 2017; Gilbert-López et al., 2017; Pan et al., 2016; Passos et al., 2015). Ultrasound-assisted extraction is suggested as a cost-effective method that can improve the extraction of phenolic compounds from macroalgae (Kadam et al., 2015). Subcritical water hydrolysis is a method that successfully extracted proteins and carbohydrates from *C. vulgaris* and proteins from *Scenedesmus* sp. However, a recent report investigated thermodynamics and kinetics of amino acids produced from *Nannochloropsis* sp. via protein hydrolysis and found that the highest amino acids (~44% DW) were obtained at 260°C for a 20 min reaction time, however, early decomposition of amino acids was evident (Zainan et al., 2019). Alternatively, water and acid hydrolysis methods were reported for extraction of phytohormones (EL Boukhari et al., 2020). Acid hydrolysis methods are also reported for extraction of fucose containing sulphated polysaccharides, whereas the commercially available biostimulant from *A. nodosum*, AZAL5 R, is extracted via acid hydrolysis. Extracts obtained via acid hydrolysis contain a high amount of free amino acids and lower soluble peptides. Most of the commercial macroalgae extracts such as Maxicrop, Seasol, and Acadian are produced via an alkaline hydrolysis method, where complex polysaccharides are broken down into small oligomers and novel compounds are produced (not initially present in the algae) via interaction of alkali and algal metabolites (EL Boukhari et al., 2020; Shukla et al., 2019). The aggressive nature of both acid/alkali hydrolysis usually results in partial or complete degradation of the amino acids tryptophan, cysteine, serine and threonine, where most of the amino acids were reported to be converted from bioactive L to D-form with no biological activity (Colla et al., 2017).

Enzymatic hydrolysis could be an eco-friendly and efficient alternative in preserving the bioactivity of molecules in the algal extracts. It has been reported to better preserve L-amino acids compared to other methods (García et al., 2012). Moreover, the selection of a specific enzyme/cocktail and its concentration along with optimal pH and temperature conditions allow selective extraction of specific compounds. A range of cell wall degrading enzymes (cellulolytic and proteolytic) are available where gentle conditions (<60°C) result in a higher peptide to free amino acids ratio and higher L-amino acids as opposed to that of acid/alkali hydrolysis methods (Colla et al., 2017). A recent report investigated the use of cellulases to improve the protein yield of macroalgae *Macrocystis pyrifera* and *Chondracanthus chamissoi*, where yields of 74.6% and 36.1% respectively were

reported (Vásquez et al., 2019). However, longer reaction times and higher cost of enzymes are major bottlenecks to enzymatic hydrolysis being commercial. A cascade approach involving the integration of several extraction methods is gaining momentum and holds potential in exploiting the algal biomass. For example, a combination of enzymatic hydrolysis with alkali extraction (Mæhre et al., 2016) and ultrasound pre-treatment with acid and alkali hydrolysis (Kadam et al., 2017) were reported as the most efficient methods for the extraction of proteins from macroalgae. A cascade process has been also suggested for the production of a microalgal biostimulant plus lipid extract (Oancea et al., 2013). The steps of the process are: 1) cell walls lysis by pressure homogenization and hydrolysis with lytic enzymes, 2) separation of phytohormones, osmoprotectants, free amino acids and soluble carbohydrates by ultrafiltration, 3) extraction of lipids from ultrafiltration retentate, 4) enzymatic hydrolysis of protein from defatted retentate, 5) mixing protein hydrolysate with ultrafiltrate retentate resulting in a microalgae based plant biostimulant (Oancea et al., 2013).

After disruption, fractionation and isolation are necessary to separate and determine the biological activity of specific compounds. Modified Bieleski's solvent, composed of methanol, formic acid and water (15:1:4) is reported to be a good choice for the simultaneous extraction of multiple plant hormones such as auxins, cytokinins, abscisic acid, jasmonic acid and others. Such a solvent prevents enzymatic degradation of hormones without extracting any lipid moieties. Alternative solvent systems reported for the simultaneous extraction of plant hormones include acetonitrile, methanol/water and propanol/water/hydrochloric acid (Du et al., 2012). Auxins and cytokinins are soluble in liquids with a high pH so alkaline extraction is recommended, where antioxidants can be added to the extraction process to prevent the oxidation. For cytokinins extraction from plants, modified Bieleski's solvent is reported to be suppressing the dephosphorylation of cytokinin mononucleotides and is considered to be superior to other solvents (Hoyerová et al., 2006). Likewise, ethanol for GAs and methanol, methanol/water and methanol/chloroform are reported for extraction of BRs (Du et al., 2012). Sulphated polysaccharides are extractable with large volumes of hot water, dilute acid, or dilute alkali. Fractionation is necessary because of the extraction complexity. Liquid-liquid extraction based on the polarity principle is a widely used method for the fractionation of complex mixtures into specific compounds. High polarity compounds (amino acids) may be extracted with n-butanol, whereas lipophilic compounds (fatty acids, terpenes, hydrocarbons) can be partitioned using CCl_4 (Górka et al., 2015). Overall, different methods of disruption and extraction can be selected depending on the desired end product and its function.

5. Application type and timing

The application type and timing of application of the biostimulants can have a significant effect on the end crop. Algal biostimulants can be applied fresh or dried to the seeds, soil, leaves or roots in both liquid and solid (powders, pellets) forms (Arnau and Richard, 2016; Drobek et al., 2019; Renuka et al., 2018). Biostimulants can be applied regularly during the whole vegetative period or proactively such as before, during or after stressed conditions (Drobek et al., 2019) and the effectiveness to counteract the stressful condition depends on timing and mode of action (Bulgari et al., 2019). Anti-stressor compounds such as proline or glutamic acid can be applied during stress whereas compounds that activate bioactive compound biosynthesis must be applied before stress occurs. Timing also depends on the crop species. The dose of algal biostimulant has been extensively reviewed and is important as this can help to avoid waste, high production costs, and unexpected results (Bulgari et al., 2019). The use of liquid concentrates are recommended for fast results, however, the benefits may not be long-lasting so repeated applications may be needed. For longer-lasting effects, macroalgae meal or pellets of microalgae should be considered, however, this incurs a higher cost (Yuvaraj and Gayathri, 2017). Another option is to apply biostimulants via a carrier such as manure or other fertilisers (Renuka et al., 2018). Standardisation and optimisation of the quantity of biomass applied is essential before application at field scale. However, this can vary depending on the soil composition, species of microalgae, and biomass characteristics (Renuka et al., 2018). There are lots of different terminologies for application methods and here we categorise them into a foliar application, soil application, seed soaking, hydroponics and carriers.

Foliar application is an environmentally friendly method whereby the extract containing biostimulant bioactives is applied to the leaves, usually by spraying. It is advised that spraying takes place during the morning when stomata pores are open and under high humidity conditions, if possible, to increase the permeability and uptake of the products (Chiaiese et al., 2018). Foliar spray is beneficial as it has direct effects on the plant

especially if extracts are rich in phytohormones. Spraying supernatant, leachate or compost tea of algal formulation is also possible. The mode of application is dependent on the purpose of the biostimulant and the type of crop and may require sophisticated equipment which could add to the cost, however, with the advents of drones, it may not be such a hurdle (Renuka et al., 2018). Foliar application of low concentrations of macroalgal biostimulants has demonstrated higher growth, yield, mineral content, biochemical constituents, antifungal properties and fruit or tuber initiation compared with controls in many different crops. It has also been shown to protect the plant against biotic and abiotic stresses (Drobek et al., 2019; Sharma et al., 2014). Examples of successful foliar application of microalgae are noted here. Regarding optimal dosage, 50% (*v/v) C. vulgaris* extract in water as a one-time foliar application (after 25 days of sowing wheat seedlings) was superior to micronutrients, resulting in 140% and 40% gain in yield and grain weight respectively (Shaaban, 2001). Likewise, 50% (*v/v*) *C. vulgaris* extract in water was reported as the optimal dose for both wheat and grapevine, however, application rates required are high compared to other application modes (Arnau and Richard, 2016). In addition, foliar application of *Chlorella* was reported to improve the stomatal functioning and anti-transpiration of crop plants (Li et al., 2014). The influence of foliar dosage treatments of Spirufert® (contains *Arthrospira* spp.) was investigated for the overall growth and yield of aubergine plants, where low concentrations (10 g L^{-1}, applied five times before flowering) resulted in greater fruit yield whereas high concentrations (45 g L^{-1}) increased vegetative growth but reduced aubergine yield (Dias et al., 2016). These findings reiterate the finding that lower concentrations have a greater effect than higher concentrations. The foliar application of polysaccharides extract (3 g L^{-1}) from *A. platensis* improved the plant size, root weight, node size and node numbers of both tomato and pepper plants (Elarroussi et al., 2016).

Soil application is when biostimulants are applied directly onto the soil to enhance soil activity, where nutrients from the product are released slowly via mineralisation caused by the soil bacteria. Mineralization can take 20-30 days if using whole biomass (Renuka et al., 2018). Whole macroalgae biomass or meal has to be applied to the soil before planting which has some limitations in terms of acquisition and transportation (Drobek et al., 2019). Whole microalgae biomass is easier to apply as it is already in a liquid or powder form. However, it is important to note that whole biomass applications are usually biofertilizers, as opposed to biostimulants. Biostimulants containing humic substances and nitrogen compounds are often applied directly onto the soil. Soil application mainly affects the structure of the root and enhances the roots ability to absorb nutrients (Drobek et al., 2019). Soil application of cyanobacteria on rice crops was investigated either as a five species mix or as a single species. In general, the mixed inoculum increased grain yields up to 20.9% and straw yield up to 18.1% (Paudel et al., 2012). It has also been demonstrated that rice seed germination, root and shoot growth, rice grains weight and protein content were improved using soil application of cyanobacteria due to root-promoting hormones such as auxins, cytokinins and gibberellic acid (Ronga et al., 2019). Such growth promoters can stimulate the growth of the soil microbial populations and crop development. When biostimulants were administered once during the occurrence of a strong stress factor, soil application of the biostimulant was found to be less effective than foliar application (Drobek et al., 2019). Foliar application results in rapid plant response and is usually recommended where a short-term response is desired, whereas soil drench application is more suitable when long-term effects are desired. This was recently demonstrated using omics approaches where the biostimulant action of plant-derived protein hydrolysates (foliar spray or substrate drench) was evaluated on tomato plants under drought conditions (Paul et al., 2019; Sestili et al., 2018). Both treatments had a positive impact on the plants compared to controls, however, under drought conditions, drench application was more effective than foliar application.

Seed soaking (or priming) is when plant seeds are soaked (18-24 hrs) in water containing a low concentration of biostimulant before sowing/germination. Macroalgae have been tested extensively for such applications with results that enhanced seedling vigour, chlorophyll content, reduction of harmful seed microflora, increased levels of plant defence enzymes and faster emergence in many crops (Sharma et al., 2014). In the case of microalgae, seeds soaked in supernatant and extract (0.75 g L^{-1}) of green alga *Acutodesmus dimorphus* resulted in faster seed germination rates (2 days earlier than the control) (Garcia-Gonzalez and Sommerfeld, 2016). Alternative application modes such as hydroponics (growing plants without soil) can contribute to the sustainable production of crop plants through the adoption of environmentally friendly efficient growing conditions (Sharma et al., 2014). Hydroponic co-culturing of *C. vulgaris* or *S. quadricauda* with tomato plants with the addition of a digestate from an agricultural and livestock farm was investigated to evaluate the

effect on algae and/or plant growth (Barone et al., 2018). With the addition of digestate, the microalgal growth was improved by 32.5% for *C. vulgaris* and 30.4% for *S. quadricauda.* The tomato plants benefitted from the microalgae, but the combination of algae and digestate was less effective than the algae alone.

The use of a carrier for the application of living algae has also been suggested (Renuka et al., 2018). This method allows organisms to grow once applied to the field. The benefit is that only a small inoculum is required therefore reducing the cost. The disadvantage is that if the soil is used as the carrier, there is a risk of contamination of the algae and field applications might be expensive. To find safe and sustainable options, different types of carriers such as wheat straw, paddy straw compost, vermiculite, clay, animal waste, and agricultural wastes have been investigated and are found to be suitable for the growth and establishment of cyanobacteria and microalgae (Renuka et al., 2018). These carriers are worth considering as they remediate wastes, however, the use of waste brings other contamination risks such as the presence of antibiotics and pharmaceuticals which have been reported to accumulate in plants (Pan and Chu, 2017). The inoculation of microalgae in animal waste could be useful, as microalgae can degrade certain pharmaceutical compounds (Yu et al., 2017). Therefore, there is scope for the use of municipal wastes as a carrier for microalgae application as biofertilizers or biostimulants. In terms of other constraints, grazers in the field could be a problem if they prevent the proliferation of the microalgae. In such cases, the use of plant-based pesticides (phytoextracts of neem, tobacco or bel) which are more environmentally friendly than chemical-based products could be incorporated into the carrier (Jha and Prasad, 2005). There is evidence for a significant increase in rice yield and decrease in grazer populations with the use of such plant-based pesticides (Jha and Prasad, 2005). Likewise, biofilms using cyanobacteria as matrices are also reported to prevent the attack of grazers, as mucilage offers protection and symbiotic association of cyanobacteria with soil fungi while other microbes produce hydrolytic enzymes against grazers (Prasanna et al., 2011). Henceforth, selection of carrier material is an important aspect to consider, and an ideal carrier should be inexpensive, easily available, non-toxic to plants and more importantly, should have good moisture-holding, pH buffering and adhesion capacity (to bind to seeds) (Mahapatra et al., 2018).

Chitosan, a polymer of glucosamine, has been developed recently as a key category of biostimulants in the agricultural field owing to its antifungal properties and for enhancing tolerance of plants to abiotic stresses (du Jardin, 2015). Algae do not produce chitosan, however, novel strategies such as co-application of algal extract and chitosan are emerging. For example, the combination of macroalgae extract and chitosan was more effective in enhancing the activity of various defence genes and enzymes in wheat seedlings and reduced the severity of *F. graminearum* infection in leaves (Gunupuru et al., 2019).

6. Current market and regulations

To enhance food security, organic farming and the shift towards sustainable agricultural practices, government authorities across the world are encouraging the use of biostimulants, leading to an increase in innovations and new product development. The global biostimulants market size is expected to reach $5.5 billion by 2027, exhibiting a compound annual growth rate (CAGR) of 12% during the 2019 to 2027 forecast period ("Transparency Market Research," 2019). As of 2016-2017, the biostimulant market is broadly dominated by different segments such as by product (acid-based (humic substances)) > extract-based (macroalgae) > others), crop type (cereals and grains > fruits and vegetables > turf and ornamentals > others (forage and plantation crops)), application method (foliar > soil > seed) and by region (Europe > North America > Asia pacific > LAMEA) ("Transparency Market Research," 2019). Today, macroalgae extracts and humic substances share ~70% of the global biostimulant market, whereas the share of microalgal biostimulants is negligible. Macroalgae extracts are currently a dominant category of the plant biostimulant segment with a predicted market of €894 million by 2022 (EL Boukhari et al., 2020). For example, *A. nodosum*, *Lithothamnion calcareum* (Rhodophyta), *Macrocystis pyrifera*, *Durvillaea antarctica (*Phaeophyceae*)*, and *E. maxima* are all extensively used in commercially available products for agricultural use as biostimulants (Khan et al., 2009), where the current market is dominated by key brands such as Göemar, Valagro and Kelpak (Dmytryk and Chojnacka, 2018).

In terms of microalgal products, biostimulants and bioplastics are the top emerging trends in Europe (Rumin et al., 2020). From the global biostimulant market, Europe acquires ~30% of revenue share (Arnau and

Richard, 2016). Non-microbial plant biostimulants such as protein hydrolysates, humic acids and macroalgae extracts are organic and are expected to account for >50% share of the European biostimulants market (EBIC, 2016), as around 8.5 million hectares of European land was treated with biostimulants in 2016. Globally, in 2018 the potential economic value of microalgae-based biostimulants was €0.9 billion (Barsanti and Gualtieri, 2018) and is predicted to be worth €2.5 billion by 2021 (Market Research, 2016). Microalgal biostimulants are estimated to cost €10-80 per kg of dry weight and treatment costs can be €100-600 per hectare depending on the application rate and the number of applications (Arnau and Richard, 2016).

Biostimulants face competition from synthetic growth promotors so the value of biostimulants from algae needs to be assessed to ensure that the production of specific compounds is viable and affordable for farmers (EL Boukhari et al., 2020). The 2018 selling price for raw microalgal biomass was €1000 per tonne but for polysaccharides extracted from microalgae, it was €10,000 per tonne (Barsanti and Gualtieri, 2018). In 2015, Ferticell microalgal biostimulants from the producer Agroplasma was reported to have a sale price of €1300-1500/tonne (Voort and VulstekeSaut, 2015). Therefore, there is a higher value in extracting specific products compared to using the whole biomass, although this does incur higher costs and increases production time. To fully exploit the commercial potential of microalgal biotechnology, bottlenecks in biological, agronomical, economic and technological areas must be addressed (Arnau and Richard, 2016). Recently, many industries in Spain (Agroplasma, AlgaEnergy, Agrialgae, Allgrow and Biorizon biotech), Turkey (Mikroalg Inc. and MCT Tarim Ltd.), USA (AgroValley Inc.), Hungary (Natur Agro) and India (Soley Biotech, Hindustan bioenergy Ltd.) advanced their research and investments in commercialising microalgal biostimulants and biofertilizers, where *Arthrospira*, *Chlorella*, *Scenedesmus*, *Haematococcus* and *Nannochloropsis* extracts have mainly been explored so far.

Farmers and consumers are willing to produce and consume safe and organic products, however, the market for microalgal biostimulants lacks credibility and is not well established due to reasons such as lack of research, lack of standard operating procedures to produce products, longer duration (3-5 years) for product development, few patents, reproducibility of lab results to the field is difficult and bottlenecks in international trading due to stricter and complicated regulations which vary between countries. Regulators aim to support sustainable agriculture by integrating safe and ecological considerations into regulations, however, there is a need for policy to define the economic and environmental benefits in terms of objectives and endpoints as there is a lack of consistency and rigorous regulation for the use of biostimulants (Arnau and Richard, 2016; du Jardin, 2015; EL Boukhari et al., 2020). The EU is the first legislative organisation to recognise plant biostimulants as a separate group of agricultural inputs (EL Boukhari et al., 2020). In Europe, Fertilizers Europe, EBIC, the European Crop Protection Association (ECPA), and the Bio-based Industries (BBI) Consortium are the key organisations that are involved in streamlining the guidelines and establishing the market and opportunities for biostimulant products to make agriculture more sustainable and resilient. EBIC provides an engagement platform for industries and key stakeholders to address key issues as they raise, EPCA deals with the pesticide industries whereas, BBI is a public-private partnership for the development of sustainable bio-based industries in Europe. Existing regulations for other products cannot be transferred to biostimulants because of the diversity of products (including microbial and non-microbial products), their effects and the interactions among biostimulant components and with other systemic elements such as weather, soil and crop type and soil microbiome (Ricci et al., 2019).

Until 2019, three key valid regulations were supervising the European market of products for plant treatment: Regulation (EC) No 2003/2003 of October 13, 2003 (relating to fertilizers); Directive 2009/128/EC of October 21, 2009 (for sustainable use of pesticides) and Regulation (EC) No 1107/2009 of October 21, 2009 (for placing plant protection products on the market) (Dmytryk and Chojnacka, 2018). Biostimulants fall under the regulations on fertilisers and are excluded from the regulatory framework relating to pesticides as they do not have any direct action on pests (EBIC, 2016). From a strict regulatory point of view, as biostimulants influence the life processes of plants by mechanisms other than direct nutrition, they are often referred to as a plant protection product (PPP's), as the Regulation (EC) 1107/2009 on plant protection products applies to all categories of biostimulant (du Jardin, 2015). However, due to the lengthy and costly process involved in placing the product under PPP's, most industries prefer the fertiliser route. In 2019, The Fertilising Products Regulation (FPR) (EU) 2019/1009 defined a plant biostimulant as "a product the function of which is to stimulate plant

nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: (a) nutrient use efficiency, (b) tolerance to abiotic stress, (c) quality traits, or (d) availability of confined nutrients in the soil or rhizosphere" ("Eur. Comm.," 2019). The regulation also specifies that a plant biostimulant "shall have the effects that are claimed on the label for the plants specified thereon". Consequently, the justification of the agronomic claim is important to allow it to be placed on the EU market. In this regard, algae-based products might face a challenge, as results reported in field-based application can be variable. A recent review outlined a guide underlying general principles to justify plant biostimulant claims (Ricci et al., 2019).

Generally, both macroalgae and microalgae fall within the same legislation and are not included in the category of microbial plant biostimulants and are therefore non-microbial plant biostimulants. According to the regulation non-GMO algae can be used as a fertilising product for plants or mushrooms to grow in as long as specific contaminant levels (metals and bacteria) do not exceed the specified values. It also allows algae (excluding cyanobacteria) to be used that has been processed via cutting, grinding, milling, sieving, sifting, centrifugation, pressing, drying, frost treatment, freeze-drying or extraction with water or supercritical CO_2 extraction. Based on the current European legislation, EBIC and the creation of BBI, there are great opportunities in exploring the full potential of microalgal biostimulants for sustainable agricultural practices. The innovative algae-based products can be launched into the market either as a biostimulant or as a plant protection product. However, bottlenecks still exist concerning organic certification, labelling and control due to the EU Regulation 2018/848 on fertilising products ("Eur. Comm.," 2018). It allows the use of nutrients from a non-GMO algae-based product that can be derived from or can contain digestate, provided digestate is sourced from plant or algal digestion (excluding cyanobacteria). However, the algae biomass grown on digestate and the digestate itself cannot be used in the feed chain if sourced from the anaerobic digestion of animal by-products or derived products falling within the scope of Regulation (EC) No 1069/2009 ("Eur. Comm.," 2009) and for which no endpoint in the manufacturing chain has been determined. Currently, a procedure of authorisation of nutrients of animal origin is not available in organic production, and therefore there is no way to be included in the restrictive list of external inputs allowed in organic production. For example, protein hydrolysate sourced from animal by-products (Corte et al., 2014) will not be allowed to enter into the food chain, even if the product has been assessed and proven to be safe in terms of genotoxicity, ecotoxicity and phytotoxicity. In such cases, the European Food Safety Association should be consulted for guidance and technical assistance on making an authorisation application for novel routes/products developed for use and disposal of animal by-products that ensure the safety of the feed chain. With regards to feed containing manures, this is strictly prohibited, however, there is no specific guidance available on the use of manure-derived algae for feed purposes.

7. Future perspectives

7.1 Bioremediation of waste resources and development of sustainable circular economy/ biorefinery approaches

The commercial reality of bioactives from algal biomass is mainly constrained by the high production costs, thereby requiring integration of waste resources (as low-cost/free nutrient input) and development of a sustainable biorefinery approach to offset this cost (Butler et al., 2020; Kapoore et al., 2018). The key waste nutrient resources that can be explored include wastewater streams and digestate from AD industries. Circular economy and reuse of nutrients from waste sources is a topical area for scientists and businesses. The environmental and economic implications of this are far-reaching, as it will see a reduction in negative discharges to the environment, reduction in GHG emissions and free waste materials turned into a cost-effective, multi-tasking and environmentally friendly biostimulant to improve growth and yield of crops. This will also improve worldwide food security. The successful growth of microalgal biomass on wastewater streams and later potential biostimulant activity of such biomass on crops have been demonstrated (Mahapatra et al., 2018; Renuka et al., 2018). The main wastewater streams covered include domestic, industrial and agricultural wastewater, whereas other potential streams are wastewater from piggery, aquaculture, soybean processing, potato processing and carpet mill effluent. The utilisation of such wastewater streams for microalgae cultivation will result in the remediation of excess nutrients which includes not only C, N and P but also heavy metals and certain pharmaceutical compounds. Moreover, on-site cultivation facilities will drastically reduce transportation

costs. In contrast, the major bottlenecks associated with the use of such wastewater streams are; risk of contaminants (heavy metals, pathogens, chemical, pesticides and pharmaceuticals), barriers in maintaining monoculture due to cross-species/bacterial contamination and finally seasonal and nutrient load variation affecting growth profiles and the end product composition of algal biomass.

An alternative promising waste nutrient resource for the production of algal biomass is digestate from anaerobic digesters (Stiles et al., 2018). The worldwide AD biogas industry is expanding with a resultant surplus of waste nutrients (digestate) in some areas that could be harmful if released uncontrolled back to the environment (as it is or even as a land-fertiliser) contributing to the eutrophication of water bodies. A solution to circumvent the possibility of eutrophication is the creation of an improved biostimulant product with a higher value. For example, growing microalgae on digestate at a large scale can bioremediate excess nitrogen and phosphorus compounds and this will eventually lead to the development of innovative sustainable agricultural biostimulant products that will open new market opportunities for both the newly developing AD biogas industry and the biostimulant industry (Fuentes-Grünewald et al., 2021). The successful growth and remediation potential of microalgal biomass grown on digestate was recently demonstrated (Jiang et al., 2018), where *C. vulgaris* and *S. obliquus* were capable of removing 57.8% and 51.6% of ammonia and 86.8% and 76.6% of phosphates respectively from a liquid digestate containing 473 mg L^{-1} ammonia and 183 mg L^{-1} phosphate. Currently, ALG-AD is an Interreg NWE funded project (2017-2021) which aims to develop novel technology to take excess waste nutrients produced from anaerobic digestion of food and farm waste to cultivate algal biomass for animal feed and other products of value ("ALG-AD," 2020). However, to our knowledge, no published reports exist on using digestate-grown microalgae as biostimulants and this needs to be explored on field-based application. Integration of such waste streams in microalgal biotechnology will create a circular economy; closing the loop within local industries by using waste products to create new, valuable products i.e biostimulants. It is high time to implement such sustainable circular economy approaches worldwide to prevent loss of energy and resources.

Fig. 1

The algal biorefinery approach is very well defined in the literature, however, this has been rarely put into practice (Butler et al., 2020). Biorefinery involves separating the algal biomass into multiple fractions that can be used independently to permit full utilisation of the biomass. This allows the generation of co-products so economic viability, energy efficiency and waste management are enhanced (EL Boukhari et al., 2020). There are many microalgal biorefinery concepts in the literature, for example, freeze-thawing and membrane filtration were used to separate polysaccharides, proteins and phycoerythrin from *Porphyridium* species (Marcati et al., 2014); proteins, lipids and carbohydrates from *S. obliquus* (Ansari et al., 2017b); EPA, chrysolaminarin (carbohydrate) and fucoxanthin from *P. tricornutum* (Gao et al., 2017); EPA, zeaxanthin, β-carotene, exopolysaccharides and phycobiliproteins from *Porphyridium purpureum* (Coward et al., 2016); and EPA and high-value proteins from *Nannochloropsis* sp. (Chua and Schenk, 2017). Likewise, polysaccharides, proteins and antioxidants were separated from *Chlorogloeopsis fritschii* (Balasundaram et al., 2012), whereas *Tetraselmis suecica* was disrupted completely, using high pressure homogenisation and a high temperature, ultrafiltration was then used to separate firstly starch and pigments (100kDa), then proteins (10kDa), with sugars remaining in the permeate (Safi et al., 2014). Recently, a biorefinery process was proposed for around 66% valorisation of microalgal biomass, using enzymatic hydrolysis first for extraction of proteins (for amino acids and peptides), then carbohydrates using thermochemical hydrolysis under mild conditions (for bioethanol); lipids (direct transesterification to produce biodiesel) and finally anaerobic digestion of residual biomass (for biogas production) (Figueroa et al., 2018). Likewise, macroalgal biorefinery concepts have recently been reviewed (Zollmann et al., 2019). However, it is important to note that most of the proposed biorefinery schemes are not techno-economically feasible and require validation and techno-economic assessment at pilot scale. More importantly, we are not aware of any proposed microalgal biorefinery scheme tailored to the production of plant biostimulants.

While developing the biorefinery platform, there should be more emphasis on; 1) selecting the correct organism/consortium with the most commercial potential; 2) mild disruption of cells to avoid damaging products; 3) the use of low cost and energy equipment for separation of products. It has been also advised that

the most valuable compounds must be extracted first to avoid losses, and that mild conditions must be used in each step to prevent decomposition of the remaining compounds. Integration of circular economy, waste streams and a biorefinery approach, as highlighted in Fig. 1, could enable zero-waste agricultural societies, however, this requires further research and investment into such processes.

7.2 High-throughput phenotyping and -omics approaches

Algae can adjust their metabolism according to surrounding growth conditions and this metabolic flexibility can be exploited in industrial biotechnology with genetic and metabolic engineering when compared to other photosynthetic organisms. However, the advancement of applications in agriculture is limited by the lack of scientific evidence on metabolic pathway regulation particularly for biostimulatory compounds (Chiaiese et al., 2018). However, as algae contain several bioactives, unravelling the possible mode of biostimulant action of specific bioactives in an extract at the metabolic and genomic level is often a difficult task (EL Boukhari et al., 2020). Moreover, this is further complicated by factors such as a) genetic variability of algal species, plants and rhizosphere microbiomes; b) synergistic and antagonistic interactions between biostimulants/algae and microbiomes resulting in manipulation of rhizosphere microbiomes and c) the nature and severity of stress factors (Bulgari et al., 2019). These grey areas remain largely unexplored and to our knowledge, very few reports investigated the underlying mode of action of biostimulants using high-throughput phenotyping (Paul et al., 2019; Povero et al., 2016), metabolomics (Elarroussi et al., 2016; Ertani et al., 2014; Farid et al., 2019; Nair et al., 2012; Paul et al., 2019; Povero et al., 2016; Rachidi et al., 2020) and transcriptomics (Goñi et al., 2016; Jannin et al., 2013; Nair et al., 2012; Povero et al., 2016; Wilson et al., 2015). Likewise, a novel complex multi-trait high-throughput screening method was developed recently (Ugena et al., 2018) for identification of novel biostimulants and characterisation of their mode of action in *Arabidopsis* germination and rosette growth under salinity, where it was claimed that such an approach can be used to classify the mode of action of biostimulants as either plant growth promoters/inhibitors or stress alleviators or combined action. Overall, such biotechnological improvements are also essential to isolate "novel strains" with improved biostimulant yield and to underpin the interaction between microalgae, rhizosphere microbiome and crops (Fig. 1). Genome sequencing techniques will help to define the gene networks controlling growth, while -omics approaches allow identification of regulatory points of cellular pathways, thus enabling future manipulation of key metabolic step, thereby improving the biostimulant potential of algal extracts. This will also give a clue for future genetic modifications (GM) of strains to enhance the productivity of these bioactive metabolites, provided there are no regulatory barriers in place. Alternatively, the forward genetic approach might be helpful in such scenarios, as it avoids the GMO regulatory route, where physical methods (UV-light, γ-rays, X-rays) or chemical mutagens (N-Methyl-N'-nitro-N-nitrosoguanidine or ethyl methanesulfonate) can be used to induce mutagenesis in microalgal strains (Benedetti et al., 2018).

7.3 Species (marine or freshwater) selection and techno-economic aspects

In contrast to macroalgal biostimulants, which are sourced from marine water, research on biostimulants from microalgae has largely focussed on freshwater species, although some marine species such as *Phaeodactylum, Dunaliella, Nannochloropsis* and *Tetraselmis* have been investigated (Tables 2-5). The extracts obtained from both marine and freshwater microalgal species are found to have similar biochemical compositions and therefore possess similar biostimulant activity on plants (Table 1-5). Recently, biostimulant properties of macroalgae, microalgae and cyanobacteria species were found to be similar, independent of their water sources (Colla and Rouphael, 2020; Mutale-joan et al., 2020). Moving forward, it is essential to consider differences in cultivation conditions/challenges between freshwater and marine species of microalgae and their implications on techno-economic aspects. The benefit of using freshwater species could be that production costs are lower compared to marine species because washing will not be required at the concentration stage to remove salt which would negatively affect land crops. In cases where marine microalgal species are considered, the removal of salt will need to be evaluated as part of the techno-economic assessment. Additionally, the production of marine species relies upon a supply of seawater thereby limiting production to coastal areas or incurring costs for transportation or artificial seawater. Contrastingly, it can also be considered that excessive use of freshwater can lead to water scarcity which can influence the water purchase costs depending on the location (Thomassen et al., 2016). This could compromise the success of microalgal cultivation in areas where water use will be prioritised for crops rather than biostimulant production. However, if the biostimulant effect

is drought resistance this could allow algal production to be prioritised, provided membrane filtration technologies are developed and integrated for water recycling (post-harvesting) and utilising waste-water (for cultivation). Irrespective of their water sources, critical consideration should also be given to the influence of cultivation conditions such as harvesting stage, season, light, temperature, pH, nutrients (replete/deplete) and CO_2 on the ability of both fresh and seawater species to accumulate biostimulatory compounds.

The biostimulant market is driven by increased customer attention to enhancing the premium value of crops. Among the types of plant growth regulators, gibberellins account for the largest market share, followed by cytokinins and auxins (Fernández et al., 2021). Additional algal metabolites with biostimulant potential can be very specific and may carry a small market volume, atleast for the coming short-term period. Increasing the scale of production for such products can saturate the market and reduce the market price hence, this should be avoided (Thomassen et al., 2016). The incorporation of a biorefinery approach can allow flexibility so that production can continue and adjust according to demand and saturation. The final selling price of microalgal biostimulants will depend on many factors. For example, the most important crucial parameters in reducing the time-to-market window and establishing economically and environmentally feasible value chains are cultivation conditions, downstream processing costs, the content and price (Thomassen et al., 2019). Furthermore, value chains must be optimised for photobioreactor (PBR) cultivation conditions, sterilisation and drying techniques, automation and minimal maintenance (Arnau and Richard, 2016). Overall, an improved understanding of economic aspects with the use of lifecycle analysis (LCA) and techno-economic assessment (TEA) tools in the real options framework should be carried out. These tools allow identification of the areas that require the most investment which can help to pin-point areas that require more research and development. TEA interactions should also integrate environmental assessments to allow identification of critical parameters for increasing the economic profitability and lowering the environmental impacts (Thomassen et al., 2016).

7.4 Prospects and challenges summary

The implementation and commercialisation of microalgal biostimulants has lagged significantly compared to macroalgae due to several challenges, which require further attention (EL Boukhari et al., 2020). For a successful route to the commercialisation of microalgal biostimulants, the following aspects as summarised in Fig. 2, need to be considered carefully. In the case of microalgal biotechnology, research should focus on; 1) Efforts in terms of identification and selection of optimal conditions and commercial microalgal strains or microalgal/bacterial consortia. 2) Analytical efforts to improve the metabolome coverage to identify novel biostimulant precursors or bioactives, as with the current analytical technologies only a fraction of the metabolome can be analysed (Kapoore and Vaidyanathan, 2016). 3) Implementation of biorefinery and sustainable circular economy approaches with the integration of waste streams for microalgal cultivation. 4) The identification and selection of optimal extraction techniques for the reliable extraction of microalgal biostimulants. 5) Improved understanding of the genetic and molecular biosynthetic pathways in algae or algal-bacteria consortia to identify novel biostimulant molecules/precursors and for future genetic modifications of strains. 6) The impact of seasonal variation and time of harvest on the composition of the final microalgal extract. 7) Improved understanding of economic aspects with the use of lifecycle analysis (LCA) and techno-economic assessment (TEA) tools in real options framework, prior to commercialisation.

Fig. 2

In the case of field-based studies, research should focus on the following aspects; 1) The transition of laboratory-based results to field-based application needs to be validated prior to commercialisation. 2) Validation of optimal application type (foliar/soil/seed/carrier), concentration, timing, duration of biostimulant effect after initial application and dosage frequencies required for enhanced biostimulant activity in different crops. 3) The impact of seasonal variations, co-application/interactions with other components (carriers/fertilizers/pesticides/other plant protection products), crop varieties/morphological status, soil properties, stress severity/nature/duration and composition of rhizosphere/endospheric microbiome on biostimulant activity requires thorough consideration. 4) The implementation of high throughput sequencing, phenotyping and -omics approaches for a) characterisation and development of novel biostimulants, b) exploring the underlying mode of action of specific bioactives or mixture on plant performance and c) characterising synergistic and antagonistic interactions between biostimulant, crop variety and

microbiomes/environment resulting in manipulation of rhizosphere microbiomes. The latter aspect is more important for future production of tailored formulations to support the colonisation of desirable microbial inoculants. 5) The potential co-application/blending of microalgal biostimulants with microbial biostimulants/ macroalgae extract/other commercial fertiliser to characterise the synergistic interactions and complementary functionalities between them in enhancing overall biostimulant activity in crops/rhizosphere microbiome. 6) Ensuring regulatory, intellectual property and patentability certainty between countries with special emphasis on regulatory barriers and ensuring compliance with the rules in terms of organic certification, eco-labelled products, use of GM strains and more specifically to ensure product safety when waste streams (from animal origin) are incorporated as a feedstock to produce biostimulants. 7) Altering farmers, regulators and industry perceptions towards biostimulants, as they are often regarded as "An eco-friendly alternative to traditional fertilisers", which is not the case. Lastly, biostimulants are not seen as an essential product so immediate and delayed benefits, such as resource savings and ecosystem services, should be promoted to farmers.

Conclusion

Microalgal biostimulants have an immense scope and potential to make agriculture more sustainable and resilient. Despite reports on similar biochemical composition and biostimulant activity in crops from both macro- and microalgal biostimulants, the microalgal biostimulant market lags significantly. Being metabolically flexible with high photosynthetic efficiency and with current upstream and downstream developments in microalgal biotechnology, it is possible to cultivate microalgae in a controlled fashion, tailored towards the production of specific biostimulant products. This offers a unique advantage over wild-harvested macroalgae, where standardisation and reliability of macroalgae extract is proving to be unsustainable and difficult to govern. Biostimulants from microalgae such as phytohormones, amino acids, polysaccharides, polyamines, phenolics, allelochemicals, vitamins, terpenoids and fatty acids are of key interest. Microalgae are also well known for their environmental services such as bioremediation of waste and CO_2 sequestration. Integration of such a concept along with the development of biorefinery platforms will make a significant impact within the evolving circular economy as an improved economically and environmentally sustainable agricultural product. A major bottleneck in the biostimulant industry, regardless of product type, is the underlying mode of action, however, a well-developed suite of molecular tools such as high-throughput phenotyping and -omics approaches should facilitate in isolating novel strains, exploring synergistic interactions and illustrating the underlying mode of microalgal biostimulant action. Besides, exploring the possible synergistic and complementary functionalities in a co-application route holds promise in enhancing the soil activity and crop performance. For example, a biostimulant product from Chlorophyta and a biofertilizer from Cyanophyta. Despite the promise, long-term sustainable commercial production of microalgal biostimulants will require attention to resolve specific field-based challenges. These include validation of optimal application type, timing, dosage and frequency, the influence of variable factors (season, crop varieties, soil type, interactions with other products) on biostimulant activity, regulatory barriers and finally gaining farmer's trust.

Conflict of interest

The authors declare no conflicts of interest.

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Figure captions:

Fig. 1. Potential circular economy approaches: Integrating nutrient bioremediation, high-throughput phenotyping and -omics tools for the development of microalgal biorefinery platforms towards the production of biostimulants for sustainable agricultural practices and possibly other high value products.

Fig. 2. Schematic highlighting key aspects and successful route to the commercialisation of microalgal biostimulants.

1834 **Table 1** A summary of phytohormones, their biosynthesis, physiological actions in plants and key algal sources.

Phytohormones	Biosynthesis	Physiological action	Key algae sources	References
Auxins	Via tryptamine or indole-3-pyruvic acid pathway in leaf primordia, young leaves and fruits	Cell elongation (via activation of the plasmalemmal H^+-ATPase), differentiation of phloem, apical dominance, tropisms, initiation of root formation and stress tolerance (drought, salinity and heat)	**Macroalgae:** *Ascophyllum, Ecklonia, Ectocarpus, Fucus, Laminaria, Macrocystis, Nereocystis, Sargassum, Undaria, Klebsormidium nitens, Caulerpa, Cladophora, Ulva, Polysiphonia, Botryocladia, Pyropia, Prionitislanceolata, Porphyra, Gelidium, Gracilaria, Gracilariopsis, Chondracanthus* and *Hypnea;* **Microalgae:** *Chlorella, Coenochloris, Acutodesmus* and *Scenedesmus;* **Cyanophyta:** *Synechocystis, Chroococcidiopsis, Anabaena, Phormidium, Oscillatoria, Nostoc, Chlorogloeopsis* and *Oscillatoria*	(Dmytryk and Chojnacka, 2018; Lijun, 2006; Pan et al., 2019; Piotrowska-Niczyporuk et al., 2018; Plaza et al., 2018; Stirk et al., 2002; Tarakhovskaya et al., 2007)
Cytokinins	Via biosynthesis of N6-isopentenyladenosine monophosphate from adenosine monophosphate and pyrophosphate (catalysed by isopentenyl-transferase) in root tips, leaves and developing seeds or changes in the structure of tRNA comprising *cis*-zeatin. The latter pathway involved in algae	Control cell division, promotion of protein and chlorophyll synthesis, chloroplast and vascular tissue development, bud development, shoot growth, fruit and flower development, apical dominance, development of the leaf blade and senescence and stress tolerance (drought and heat)	**Macroalgae:** *Ascophyllum, Cystoseira, Ecklonia, Fucus, Macrocystis, Sargassum, Undaria, Dictyota, Laminaria, Pyropia, Sarcothalia, Amphiroa, Corallina, Porphyras, Gelidium, Gracilaria, Gracilariopsis, Chondracanthus, Hypnea, Gigartinaclathrata, Hypnea, Ulva, Cladophora* and *Halimeda;* **Microalgae:** *Chlorella, Chlamydomonas, Desmococcus, Euglena, Myrmecia, Stigeoclonium, Mychonastes, Scenedesmus, Nannochloropsis, Nautococcus* and *Protococcus;* **Cyanophyta:** *Arthronema, Synechocystis, Chroococcidiopsis, Anabaena, Phormidium, Oscillatoria, Calothrix, Chlorogloeopsis* and *Rhodospirillum*	(Dmytryk and Chojnacka, 2018; Górka et al., 2015; Kiseleva et al., 2012; Pan et al., 2019; Plaza et al., 2018; Stirk et al., 2013; Tarakhovskaya et al., 2007)
Gibberellins	Via pathways of isoprenoid synthesis (though methylerythritol phosphate pathway) in plastids	Associated with stem elongation, initiation of seed germination via activation of α-amylase, floral organ development, influence protein biosynthesis and initiation of flowering	**Macroalgae:** *Cystoseira, Ecklonia, Fucus, Petalonia and Sargassum, Caulerpa, Ulva, Pyropia and Hypnea;* **Microalgae:** *Scenedesmus, Stigeoclonium, Gyoerffyana, Myrmecia, Nautococcus, Chorella, Scotiellopsis, Chlorococcum, Chlamydomonas* and *Nannochloropsis;* **Cyanophyta:** *Arthrospira, Anabaenopsis, Cylindrospermum* and *Phormidium*	(Bose et al., 2013; Górka et al., 2015; Plaza et al., 2018; Stirk et al., 2014, 2013; Tarakhovskaya et al., 2007)
Brassinosteroids	From campesterol via 2 pathways, either early C6-oxidation pathway and late C6-oxidation pathway	Controls division, elongation and differentiation of vascular system, promotion of ethylene production and stress tolerance	**Macroalgae:** *Ecklonia* and *Hydrodictyon;* **Microalgae:** *Klebsormidium*	(Bajguz and Tretyn, 2003; Stirk et al., 2014, 2013; Tarakhovskaya et al., 2007)
Ethylene	From methionine in stressed tissues and fruits via 1-aminocyclopropane-1-carboxylate synthase or 1-aminocyclopropane-1-carboxylate oxidase	Regulates developmental processes such as fruit ripening, opening of flowers, cell division, cell elongation, senescence of vegetative tissues and biotic and abiotic stress tolerance	**Macroalgae:** *Ecklonia, Padina, Klebsormidium, Porphyra* and *Pyropia;* **Microalgae:** species of *Chlorella and Scenedesmus;* **Cyanophyta:** species of *Arthrospira, Synechococcus, Anabaena, Nostoc, Calothrix, Scytonema* and *Cylindrospermum*	(Lu and Xu, 2015; Plaza et al., 2018; Tarakhovskaya et al., 2007; Van de Poel et al., 2016)
Abscisic acid	From carotenoids in roots and expanded leaves	Induces stomatal closure, shoots growth inhibition, protein storage in seeds with seed dormancy	**Macroalgae:** species of *Ascophyllum, Ecklonia, Saccharina, Laminaria, Ulva, Porphyra, Gelidium, Gracilaria, Gracilariopsis, Chondracanthus* and *Hypnea;* **Microalgae:** species of *Scenedesmus, Chlorella, Dunaliella, Haematococcus, Chlamydomonas* and *Nannochloropsis;* **Cyanophyta:** species of *Synechococcus, Nostocmuscorum, Trichormusvariabilis* and *Anabaena*	(Boyer and Dougherty, 1988; McAdam et al., 2016; Plaza et al., 2018; Stirk et al., 2004)
Jasmonic acid	In two steps in chloroplast and in peroxisomes from α-linolenic acid	Regulation of plant defence responses, synthesis of proteinase inhibitors, promotion of tuber formation and senescence	**Macroalgae:** species of *Fucus, Gelidium and Lithothamnion;* **Microalgae:** species of *Chlorella, Scenedesmus, Dunaliella, Euglena* and *Gelidium;* **Cyanophyta:** species of *Arthrospira*	(Dmytryk and Chojnacka, 2018; Plaza et al., 2018; Tarakhovskaya et al., 2007)
Salicylic acid	From chorismate via either isochorismate synthase (ICS) or phenylalanine ammonia-lyase (PAL) pathway	Regulation of plant defence responses during pathogenesis	**Microalgae:** species of *Scenedesmus;* **Cyanophyta:** species of *Arthrospira*	(Lefevere et al., 2020; Plaza et al., 2018; Tarakhovskaya et al., 2007)
Polyamines	Via decarboxylation of arginine or ornithine	Stress response regulation in plants and metabolic processes such as cell proliferation, differentiation and growth	**Macroalgae:** species of *Gelidium, Grateloupia, Dictyota,* and *Ulva;* **Microalgae:** *species of Chlorella, Cyanidium,* and *Euglena;* **Cyanophyta:** species of *Arthrospira*	(Kusano et al., 2008; Marián et al., 2000; Mógor et al., 2018; Srivastava et al., 2013; Tarakhovskaya et al., 2007)
Betaines	Via choline and glycine. Glycinebetaine is synthesised from choline via 2-step oxidation of choline via the toxic intermediate betaine aldehyde	Acts as an osmolyte and regulates defence mechanism against abiotic stresses such as salinity, frost and drought	**Macroalgae:** species of *Ascophyllum, Fucus, Laminaria, Laurencia, Mastocarpus* and *Chaetomorpha*	(Blunden et al., 1992; Khan et al. 2009; Sakamoto and Murata, 2002)
Rhodomorphin	-	In morphogenesis, induction of fusion of	**Macroalgae:** species of *Griffithsia*	(Waaland and Watson, 1980)

	fragments, and restoration of filament integrity

Table 2 The highest reported concentration of the phytohormones; auxins, cytokinins and gibberellins found in algae.

Phytohormones	Sub-class	Concentration reported*			References
		Per dry weight	(In) algal species		
Auxins	Indoleacetic acid (IAA)	5965 ng/g	*Scenedesmus* sp.	Microalgae	(Lijun, 2006; Piotrowska-Niczyporuk et al., 2018; Plaza et al., 2018; Stirk et al., 2002)
		110.2 ng/g	*Polysiphonia urceolata*	Macroalgae	
	Indolebutyric acid (IBA)	0.5 mg/L	*Coenochloris* sp.	Microalgae	
Cytokinins	**Total cytokinin**	21.4 nmol/g DW (~4 mg/Kg)	*Stigeoclonium nanum*		(Dmytryk and Chojnacka, 2018; Górka et al., 2015; Kiseleva et al., 2012; Pan et al., 2019; Plaza et al., 2018; Stirk et al., 2013, 2003; Tarakhovskaya et al., 2007)
	Trans-zeatin	191.37 ng/g	*Arthrospira*		
	Zeatin riboside	6.82 ng/g	*Scenedesmus*		
	Isopentenyl adenine	45561.97 ng/g	*Scenedesmus*		
	tZ	92.4 pmol/g	*Stigeoclonium nanum*		
	tZR	25.4 pmol/g	*Stigeoclonium nanum*		
	tZOG	161.1 pmol/g	*Myrmecia bisecta*		
	tZROG	4.1 pmol/g	*Nautococcus mamilatus*		
	tZ9G	59 pmol/g	*Chlorella pyrenoidosa*		
	tZRMP	1045.7 pmol/g	*Stigeoclonium nanum*		
	cZ	5339.1 pmol/g	*Chlorella minutissima*	Cyanophyta & Microalgae	
	cZR	1091 pmol/g	*Poloidion didymos*		
	cZOG	351.6 pmol/g	*Chlorella minutissima*		
	cZROG	508.7 pmol/g	*Nautococcus mamilatus*		
	cZRMP	8967.2 pmol/g	*Stigeoclonium nanum*		
	DHZ	100.4 pmol/g	*Stigeoclonium nanum*		
	DHZR	210.9 pmol/g	*Stigeoclonium nanum*		
	DHZOG	73.8 pmol/g	*Chlorella vulgaris*		
	DHZROG	57.8 pmol/g	*Stigeoclonium nanum*		
	DHZRMP	412.2 pmol/g	*Stigeoclonium nanum*		
	iP	336.7 pmol/g	*Chlamydomonas reinhardtii*		
	iPR	1232.2 pmol/g	*Poloidion didymos*		
	iPRMP	11376.3 pmol/g	*Stigeoclonium nanum*		
	Total cytokinin	0.1 to 1 mg/L	*Commercial extracts*		
	tZ	11.9 pmol/g	*Laminaria pallida*		
	tZR	0.7 pmol/g	*Amphiroa ephedraea*		
	tZOG	44.1 pmol/g	*Sarcothalia scutellata*		
	tZR5MP	6.9 pmol/g	*Laminaria pallida*		
	cZ	79.1 pmol/g	*Hypnea spicifera*		
	cZR	28.3 pmol/g	*Cladophora capensis*		
	cZOG	29 pmol/g	*Carradoeriella virgata*		
	cZROG	7.5 pmol/g	*Cladophora capensis*		
	cZR5MP	56.9 pmol/g	*Ulva* sp.		
	DHZ	0.15 pmol/g	*Halimeda cuneata*	Macroalgae	
	iP	82.4 pmol/g	*Porphyra capensis*		
	iPR	132.6 pmol/g	*Amphiroa bowerbankii*		
	iPR5MP	43.1 pmol/g	*Macrocystis angustifolia*		
	Benzyl adenine	2 pmol/g	*Dictyota* sp.		
	meta-topolin	4.8 pmol/g	*Hypnea venosa and Hypnea spicifera*		
	ortho-topolin	22.5 pmol/g	*Corallina sp.*		
	ortho-topolin riboside	3.5 pmol/g	*Laminaria pallida*		
	meta-topolin-O-glucoside	9.9 pmol/g	*Sarcothalia scutellata*		
	ortho-Topolin-O-glucoside	3.3 pmol/g	*Sarcothalia scutellata*		
Gibberellins	**Total gibberellins**	790 pg/mg	*Stigeoclonium nanum*		(Bose et al., 2013; Górka et al., 2015; Plaza et al., 2018; Stirk et al., 2014; W A Stirk et al., 2013; Tarakhovskaya et al., 2007)
	GA1	208.81 ng/g	*Scenedesmus*		
	GA3	4.1 pg/mg	*Myrmecia bisecta*		
	GA4	31.27 ng/g	*Arthrospira*		
	GA5	34.4 pg/mg	*Gyoerffyana humicola*		
	GA6	383.3 pg/mg	*Gyoerffyana humicola*		
	GA7	3.4 pg/mg	*Gyoerffyana humicola*		
	GA8	25.5 pg/mg	*Chlorococcum ellipsoideum*		
	GA9	86.3 pg/mg	*Gyoerffyana humicola*		
	GA12	443.5 pg/mg	*Myrmecia bisecta*		
	GA12ald	494.4 pg/mg	*Gyoerffyana humicola*	Microalgae	
	GA13	348.7 pg/mg	*Chlorococcum ellipsoideum*		
	GA15	3452.9 pg/mg	*Scotiellopsis terrestris*		
	GA19	5.8 pg/mg	*Myrmecia bisecta*		
	GA20	7.4 pg/mg	*Myrmecia bisecta / Gyoerffyana humicola*		
	GA24	22.3 pg/mg	*Myrmecia bisecta*		
	GA29	17.9 pg/mg	*Gyoerffyana humicola*		
	GA34	3.2 pg/mg	*Myrmecia bisecta*		
	GA44	81.9 pg/mg	*Nautococcus mamillatus*		
	GA51	609.8 pg/mg	*Nautococcus mamillatus*		
	GA53	138.5 pg/mg	*Chlorococcum ellipsoideum*		
	Total gibberellins	4746.1 pg/mg	*Scotiellopsis terrestris*		
	GA1	0.08-0.13 pg/mg			
	GA3	0.11 pg/mg			
	GA4	4.47 pg/mg			
	GA5	1.03 pg/mg			
	GA6	1.34 pg/mg			
	GA7	0.18 pg/mg			
	GA8	0.78 pg/mg			
	GA9	10.58 pg/mg		Macroalgae	
	GA13	0.28 pg/mg	*Ecklonia maxima* (stipe)		
	GA15	3.8 pg/mg			
	GA19	0.48 pg/mg			
	GA20	0.5 pg/mg			
	GA24	0.58 pg/mg			
	GA29	0.41 pg/mg			
	GA34	0.31 pg/mg			
	GA44	2.18 pg/mg			
	GA51	11.52 pg/mg			

	GA53	2.8 pg/mg

*Only highest concentration and corresponding species is reported

Table 3 The highest reported concentration of the phytohormones; brassinosteroids, ethylene, abscisic acid, jasmonic acid, salicylic acid, polyamines, betaines and rhodomorphin found in algae.

Phytohormones	Sub-class	Concentration reported*			References
		Per dry weight	(In) algal species		
Brassinosteroids	Brassinolide	548.7 pg/mg			(Bajguz and Tretyn, 2003; Stirk et al., 2014; W A Stirk et al., 2013; Tarakhovskaya et al., 2007)
	Castasterone	429.1 pg/mg	*Klebsormidium flaccidum*	Microalgae	
	Total brassinosteroids	977.8 pg/mg			
	Brassinolide	12.5 pg/mg			
	Castasterone	16.42 pg/mg	*Ecklonia maxima*	Macroalgae	
	Total brassinosteroids	28.9 pg/mg			
Ethylene	1-aminocyclopropane-1-carboxylic acid (ACC), an ethylene presursor	545.55 ng/g	*Arthrospira*	Cyanophyta	(Lu and Xu, 2015; Plaza et al., 2018; Tarakhovskaya et al., 2007; Van de Poel et al., 2016)
Abscisic acid	-	3718.25 ng/g	*Scenedesmus*	Microalgae	(Boyer and Dougherty, 1988; McAdam et al., 2016; Plaza et al., 2018; Stirk et al., 2004)
		20.74 pg/mL	*Kelpak®*	Macroalgae	
		0.03 - 0.15 µg/g	*Ascophyllum nodosum*		
Jasmonic acid	-	75.13 ng/g	*Scenedesmus*	Microalgae	(Dmytryk and Chojnacka, 2018; Plaza et al., 2018; Tarakhovskaya et al., 2007)
Salicylic acid	-	156713.72 ng/g	*Scenedesmus*	Microalgae	(Lefevere et al., 2020; Plaza et al., 2018; Tarakhovskaya et al., 2007)
Polyamines	Putrescine (free)	0.76 µg/g	*Arthrospira platensis*	Cyanophyta	(Kusano et al., 2008; Marián et al., 2000; Mógor et al., 2018a; Srivastava et al., 2013; Tarakhovskaya et al., 2007)
		134 µg/g	*Geldium canariensis*	Macroalgae	
	Spermine (free)	3.31 µg/g	*Arthrospira platensis*	Cyanophyta	
		1.81 µg/g	*Geldium canariensis*	Macroalgae	
	Spermidine (free)	0.67 µg/g	*Arthrospira platensis*	Cyanophyta	
		0.75 µg/g	*Grateloupia doryphora*	Macroalgae	
Betaines	Glycinebetaine	2.04%	*Chaetomorpha capillaris*	Macroalgae	(Blunden et al., 1992; Khan et al., 2009; Sakamoto and Murata, 2002)
	γ-aminobutyric acid betaine	0.22%	*Laurencia platycephala*		
	Prolinebetaine	0.08%	*Mastocarpus stellatus*		
Rhodomorphin	-		*Griffithsia pacifica*	Macroalgae	(Waaland and Watson, 1980)

*Only highest concentration and corresponding species is reported

Table 4 The highest reported concentration range of proteins, amino acids, humic and fulvic acids found in algae.

Bioactives	Sub-class	Concentration range*			References
		Per dry weight	(In) algal species		
Proteins and Amino acids	Total proteins	51 to 58%	*Chlorella vulgaris*	Microalgae	(Becker, 2007; Brown, 1991; Koyande et al., 2019; Mišurcová et al., 2014; Pangestuti and Kim, 2015)
		60 to 71%	*Arthrospira maxima*	Cyanophyta	
		28 to 47%	*Porphyra tenera*	Macroalgae	
		40 to 45% (total essential AA)	*Ulva reticulata, Ulva armoricana, Hypnea musciformis* and *Kappaphycus alvarezii*	Macroalgae	
	Arginine	5.9 to 13.5%	*Tetraselmis chui,* sp. of *Dunaliella, Chlorella* and *Scenedesmus*	Microalgae	
		9.50%	*Arthrospira platensis*	Cyanophyta	
		8.70%	*Ulva reticulata*	Macroalgae	
	Histidine	2.60%	sp. of *Nannochloropsis* and *Dunaliella*	Microalgae	
		2.20%	*Arthrospira platensis*	Cyanophyta	
		2.90%	*Caulerpa racemosa*	Macroalgae	
	Isoleucine	5.80%	*Chaetoceros gracilis*	Microalgae	
		6.70%	*Arthrospira platensis*	Cyanophyta	
		5%	*Caulerpa lentillifera*	Macroalgae	
	Leucine	11%	*Dunaliella bardawil*	Microalgae	
		10%	*Arthrospira platensis*	Cyanophyta	
		8.50%	*Sargassum vulgare*	Macroalgae	
	Lysine	9%	*Chlorella* sp.	Microalgae	
		5.10%	*Arthrospira* sp.	Cyanophyta	
		6.60%	*Caulerpa lentillifera*	Macroalgae	
	Methionine	3.20%	*Pavlova lutheri*	Microalgae	
		2.90%	*Arthrospira* sp.	Cyanophyta	
		2.70%	*Palmaria palmata*	Macroalgae	
	Phenylalanine	5.4 to 7.1%	*Chaetoceros gracilis* and *Dunaliella bardawil*	Microalgae	
		5.30%	*Arthrospira platensis*	Cyanophyta	
		5.40%	*Caulerpa racemosa*	Macroalgae	
	Threonine	4 to 5.9%	*Chaetoceros gracilis* and *Dunaliella bardawil*	Microalgae	
		6.20%	*Arthrospira platensis*	Cyanophyta	
		6.40%	*Caulerpa lentillifera*	Macroalgae	
	Tryptophan	0.86 to 2.1%	*Chlorella vulgaris, Phaeodactylum tricornutum, Nannochloropsis oculata* and *Isochysis galbana*	Microalgae	
		1.40%	*Arthrospira maxima*	Cyanophyta	
		0.71-1.65g/16g N	*Laminaria japonica*	Macroalgae	
	Valine	5.7 to 6.7%	*Pavlova lutheri* and *Chlorella* sp.	Microalgae	
		7.10%	*Arthrospira platensis*	Cyanophyta	
		6.90%	*Palmaria palmata*	Macroalgae	
	Cysteine	4.00%	*Scenedesmus* sp.	Microalgae	
		0.90%	*Arthrospira platensis*	Cyanophyta	
		0.96 to 7.48 g/16g N	*Eisenia bicyclis*	Macroalgae	
	Aspartic acid	10.50%	*Scenedesmus* sp.	Microalgae	
		8.60%	*Arthrospira maxima*	Cyanophyta	
		18.50%	*Palmaria palmata*	Macroalgae	
	Glutamic acid	13.80%	*Nannochloropsis* sp.	Microalgae	
		12.60%	*Arthrospira maxima*	Cyanophyta	
		14.60%	*Caulerpa racemosa*	Macroalgae	
	Serine	4.60%	*Scenedesmus* sp. and *Dunaliella bardawil*	Microalgae	
		5.10%	*Arthrospira platensis*	Cyanophyta	
		6.40%	*Ulva reticulata*	Macroalgae	
	Proline	8.30%	*Nannochloropsis* sp.	Microalgae	
		4.20%	*Arthrospira maxima*	Cyanophyta	
		5.10%	*Ulva reticulata*	Macroalgae	
	Glycine	7.10%	*Scenedesmus obliquus*	Microalgae	
		5.70%	*Arthrospira platensis*	Cyanophyta	
		13.30%	*Palmaria palmata*	Macroalgae	
	Alanine	9%	*Scenedesmus obliquus*	Microalgae	
		9.50%	*Arthrospira platensis*	Cyanophyta	
		8.50%	*Ulva fasciata*	Macroalgae	
	Tyrosine	4.20%	*Chlorella* sp.	Microalgae	
		5.30%	*Arthrospira platensis*	Cyanophyta	
		3.90%	*Caulerpa lentillifera*	Macroalgae	
Humic and fulvic acids	Humic acid	10%	*Pilayella littoralis*	Macroalgae	(Ghabbour et al., 1994)

*Only highest concentration range and corresponding species is reported

Table 5 The highest reported concentration range of polysaccharides, antioxidants, vitamins and enzymes found in algae.

Bioactives	Sub-class	Concentration range*			References
		Per dry weight	(In) algal species		
Polysaccharides	Total carbohydrate	40 to 57%	*Porphyridium cruentum* and *Chaetoceros gracilis*	Microalgae	(Brown, 1991; Craigie, 2011; Dmytryk and Chojnacka, 2018; Dragone et al., 2011; Elarroussi et al., 2016; Farid et al., 2019; Górka et al., 2015; Kidgell et al., 2019; Koyande et al., 2019; Llewellyn et al., 2019; Percival, 1979; Rachidi et al., 2020; Raposo et al., 2013; Rioux and Turgeon, 2015; Usov et al., 2001)
		6 to 19.4%	*Arthrospira Platensis*	Cyanophyta	
		64 to 88%	*Ulva gigantea* and *Porphyra Sp.*	Macroalgae	
	Total exo-polysaccharides	199.80%	*Dunaliella salina*	Microalgae	
	Ulvans	8 to 29%	*Ulva* and *Entermorpha*	Macroalgae	
	Sulphated galactans (agar and carrageenan)	70%	*Florideophyceae*	Macroalgae	
	Agarans	20 to 30%	*Gelidium* and *Gracilaria genera*	Macroalgae	
	Carrageenans (κ, ι, and λ forms)	30 to 80% of the cell wall constituents with ~35% sulfate groups	*Chondrus crispus*, *Gigartina* and *Furcellaria* (k- and λ), *Kappaphycus alvarezii* and *Eucheuma denticulatum* (k- and L)	Macroalgae	
	Alginate	9.2 to 40%	*Alaria marginata* and *Ascophyllum nodosum*	Macroalgae	
	Fucoidan	2 to 20.4%	*Undaria pinnatifida, Fucaceae, Laminariaceae, Saundersella simplex, Ascophyllum nodosum*	Macroalgae	
	Starch	7.0 to 45%	*Chlamydomonas reinhardtii*	Microalgae	
	Laminarin	0 to 18%	*Ascophyllum nodosum*	Macroalgae	
	Neutral sugar	40.53 to 77.52%	*Chlamydomonas reinhardtii* and *Dunaliella salina*	Microalgae	
	Uronic acid (in mole% of total polysaccharides)	2.15 to 23.95%	*Dunaleilla salina* and *Cylindrotheca closterium*	Microalgae	
		7%	*Arthrospira platensis*	Cyanophyta	
		47.10%	*Ulva lactuca*	Macroalgae	
	Sulfates (in mole% of total polysaccharides)	0.42 to 11.54%	*Dunaleilla salina*	Microalgae	
		5 to 20%	*Arthrospira Platensis*	Cyanophyta	
		2.5 to 40%	*Bifurcaria bifurcata* and *Ulva intestinalis*	Macroalgae	
Antioxidants and vitamins	Cobalamin (B12)	0.85 to 1.95 mg/kg	*Tetraselmis* sp. CS-362 and *Stichococcus* sp. CS-92	Microalgae	(Brown, 1991; Deli et al., 2014; Esteban et al., 2009; Fabregas and Herrero, 1990; MacArtain et al., 2007; Miyashita and Hosokawa, 2007; Paliwal et al., 2016; Shebis et al., 2013; Škrovánková, 2011; Venkataraman and Neelakantan, 1967; Vershinin and Kamnev, 1996)
		1.2 to 1.5 mg/kg	*Cylindrospermum muscicol*	Cyanophyta	
		0.12 to 1.3 mg/kg	*Porphyra* sp.	Macroalgae	
	Nicotinic acid (B3)	77.7 to 89.3 mg/kg	*Tetraselmis suecica*	Microalgae	
		1000 mg/kg	*Ulva* sp.	Macroalgae	
	Biotin (B7)	0.8 to 1.9 mg/kg	*Pavlova pinguise* and *Isochrysis galbana*	Microalgae	
	Pantothenic acid (B5)	9.1 to 37.7 mg/kg	*Tetraselmis suecica*	Microalgae	
		4.6 to 25 mg/kg	*Arthrospira* sp.	Cyanophyta	
	Ascorbic acid (C)	1000 to 3200 mg/kg	*Nannochloropsis* sp. CS-246	Microalgae	
		1071 to 3000 mg/kg	*Undaria pinnatifida* and *Enteromorpha flexuosa*	Macroalgae	
	α-tocopherol (E)	58.2 to 669 mg/kg	*Chlorella stigmatophora* and *Nannochloropsis* sp. CS-246	Microalgae	
		50 to 190 mg/kg	*Arthrospira* sp.	Cyanophyta	
		174 to 1320 mg/Kg	*Undaria pinnatifida* and *Macrocystis pyrifera*	Macroalgae	
	Thiamine (B1)	29 to 109 mg/kg	*Tetraselmis* sp. CS-362	Microalgae	
		34 to 50 mg/kg	*Arthrospira* sp.	Cyanophyta	
		1.3 to 50 mg/kg	*Undaria pinnatifida* and *Saccharina* sp.	Macroalgae	
	Riboflavin (B2)	25 to 62 mg/kg	*Nannochloropsis* sp. CS-246	Microalgae	
		30 to 46 mg/Kg	*Arthrospira* sp.	Cyanophyta	
		13.5 to 117 mg/kg	*Undaria pinnatifida*	Macroalgae	
	Folic acid	18 to 26 mg/kg	*Nannochloropsis sp CS-246*	Microalgae	
		0.5 mg/kg	*Arthrospira* sp.	Cyanophyta	
	Pyridoxine (B6)	3.6 to 17 mg/kg	*Stichococcus* sp. CS-92	Microalgae	
		5 to 8 mg/kg	*Arthrospira* sp.	Cyanophyta	
		0.12 to 64 mg/kg	*Laminaria digitata*	Macroalgae	
	Retinol (A)	0.25 to 2.2 mg/kg	*Tetraselmis* sp. CS-362	Microalgae	
		29.9 to 52.6 mg/kg	*Kappaphycus alvarezzi*	Macroalgae	
	B8	0.12 to 64 mg/kg	*Laminaria digitata*	Macroalgae	
	B9	456 mg/kg	*Ascophyllum nodosum*	Macroalgae	
	Ergocalciferol (D2)	0.35 to 0.45 mg/kg	*Nannochloropsis* sp. CS-246	Microalgae	
	Total carotenoids	4.85 to 7.20 mg/g	sp. of *Chlorella* and *Anikistrodesmus*	Microalgae	
		4.41 mg/g	*Synechocystis* sp. CCNM 2513	Cyanophyta	
		9.79 mg/g	*Sargassum sinclairii*	Macroalgae	
	β-carotene	140 g/kg	*Dunaliella salina*	Microalgae	
		1.03 mg/g	*Nostoc* sp.	Cyanophyta	
		43% of total carotenoids	*Gracilaria lichenoides* and *Gracilaria verrucosa*	Macroalgae	
	α-carotene	150 mg/kg	*Grasiella emersonii*, sp. of *Chlorococcum* and *Ankistrodesmus*	Microalgae	
		0.53% of total carotenoids	*Dunaliela salina*	Microalgae	
		680 mg/kg	*Synechocystis pevalekii* CCNM 2520	Cyanophyta	

		21 to 27% of total carotenoids	*Codium fragile* and *Palmaria palmata*	Macroalgae	
	Zeaxanthin	1110 mg/kg	*Monoraphidium minutum*	Microalgae	
		2.06% of total carotenoids	*Dunaliela salina*	Microalgae	
		910 mg/kg	*Synechococcus* sp. CCNM 2514	Cyanophyta	
		57-68% of total carotenoids	*Ahnfeltia tabuchiensis*	Macroalgae	
	Neoxanthin	4130 mg/kg	*Acutodesmus dimorphus* CCNM 1045	Microalgae	
		0.38 to 0.79% of total carotenoids	*Dunaliela salina* and *Euglena sanguinea*	Microalgae	
		15 to 18% of total carotenoids	*Ulva fenestrata*	Macroalgae	
	Lutein	3130 mg/kg	*Chlorella* sp. CCNM 1019	Microalgae	
	Canthaxanthin	150 mg/kg	*Monoraphidium minutum* CCNM 1042	Microalgae	
Enzymes	**Amylase**	0.112 to 0.562 u/ml	*Westiellopsis prolific*	Cyanophyta	(Al-Hussieny et al., 2019)
	Protease	3.7 to 12.983 u/ml	*Microcystis aeruginosa* and *Oscillatoria sancta*	Microalgae & Cyanophyta	
	Beta-lactamase	26.7 u/ml	*Chrococcum* sp.	Cyanophyta	
	Phosphatase	0.03 to 0.06 u/ml	*Anabaena variabilia*	Cyanophyta	

*Only highest concentration range and corresponding species is reported