



Review

Integrated microalgal biorefinery for the production and application of biostimulants in circular bioeconomy

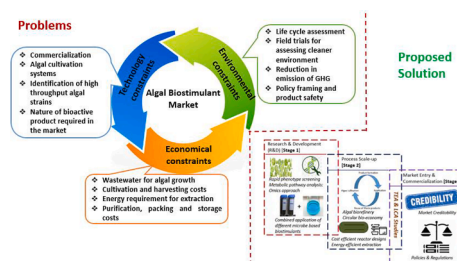
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HIGHLIGHTS

- Microalgae offers rich source of multi-functional bioactive compounds.
- Microalgal biostimulants elicit signaling pathways providing systemic resistance.
- Market opportunities and criticalities for commercialization are described.
- Enviro-economic constraints can be addressed by integrated algal biorefineries.
- Circular bioeconomy concepts increase algal biostimulants market credibility.

GRAPHICAL ABSTRACT



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ABSTRACT

Adverse detrimental impacts of environmental pollution over the health regimen of people has driven a shift in lifestyle towards cleaner and natural resources, especially in the aspects of food production and consumption. Microalgae are considered a rich source of high value metabolites to be utilized as plant growth biostimulants. These organisms however, are underrated compared to other microbial counterparts, due to inappropriate knowledge on the technical, enviro-economical constraints leading to low market credibility. Thus, to avert these issues, the present review comprehensively discusses the biostimulatory potential of microalgae interactively combined with circular bio-economy perspectives. The biochemical content and intracellular action mechanism of microalgal biostimulants were described. Furthermore, detailed country-wise market trends along with the description of the existing regulatory policies are included. Enviro-techno-economic challenges are discussed, and the consensus need for shift to biorefinery and circular bio-economy concept are emphasized to achieve sustainable impacts during the commercialization of microalgal biostimulants.

1. Introduction

The conventional food production practices utilize a huge quantity of chemicals as fertilizers/plant growth promoters, herbicides/pesticides,

thereby degrading the environment through eutrophication and increased greenhouse gas (GHG) emissions (Chandini et al., 2019). Apart from causing loss of biodiversity, these synthetic chemicals in residual amounts also gets accumulated in the crops or food products

Abbreviations: ABA, Absciscic acid; SA, Salicylic acid; ET, Ethylene; SOD, Superoxide dismutase; POD, Peroxidase; PPO, Polyphenol oxidase; PAL, Phenylalanine ammonia lyase.

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(popularly termed as bioaccumulation), and when consumed, cause detrimental health impacts (Sharma and Singhvi, 2017). The increased consumer demands for healthy and safe food products in recent times necessitates the replacement of the synthetic chemicals with bio-based molecules or microbial products termed as “biostimulants”. These bioactive components applied in small dosage increases the assimilation of nutrients, promotes overall growth and metabolism with increase tolerance of plants towards biotic and abiotic stress (Arnau, 2016; Barone et al., 2018). Broadly, the biostimulants can be categorized as microbe based biostimulants like microalgal extracts and the bacterial, yeast based compounds and non-microbe based biostimulants like macroalgal extracts, protein hydrolysates and humic acid (Kapoor et al., 2021). Since, algae based extracts (both macroalgae and microalgae) provides a wide array of biochemical components like proteins, carbohydrates and lipids along with the bioactive metabolites like phytohormones, humic acid like substances, carotenoids, vitamins etc., compared to the other microbial or non-microbial counter parts, they have received substantial attention over years (Blanke, 2016; Michalak et al., 2017). Further, these phototrophs can also sequester carbon dioxide (CO₂), thus, provide additional advantages of greenhouse gas (GHG) mitigation (Behera et al., 2019a). Compared to macroalgae, which are being widely explored over years as the source of bioactive compounds, not much research has been done on microalgal biostimulants (Kapoor et al., 2021). However, microalgae could act as a more sustainable source for biostimulant production compared to macroalgae, whose use is limited due to several underlying reasons of seasonal fluctuations and country-wise restrictions (Kapoor et al., 2021; El-Boukhari et al., 2020). Contrary to these, microalgae can be grown using waste resources and can easily be tailored to accumulate higher amounts of bioactive metabolites. Also, the comparative evaluation of biostimulatory potential of macroalgae and microalgae shows similar activity on the plant growth and its metabolism (Oancea et al., 2013). The studies on microalgal biostimulants are recently emerging mostly deriving motivation from the natural ecosystem events wherein algae present in paddy fields have rhizospheric interactions via the release of extracellular bioactive metabolites promoting nitrogen fixation (Chakdar et al., 2012) and this phenomenon has also been reported to enhance the plant defense mechanism (Gupta et al., 2013). Recent studies on the extracts of *Scenedesmus* sp., and *Chlorella* sp., *Tetraselmis* sp., *Scenedesmus* sp., *Nannochloropsis* sp., and *Isochrysis* sp., (Ronga et al., 2019) having essential exopolysaccharides, protein hydrolysates and amino acids along with crucial phytohormones, showed plant growth promoting potential of these species.

Most of the reviews in the arena published recently as in instance, the detailed summary by Chiaiese et al. (2018) and Ronga et al. (2019) have discussed the use of whole algal biomass (wet/dry) as a source of plant growth promoter with less focus over the bioactive metabolites present in microalgal extracts over the inherent plant metabolism resulting in improved yield and productivity. A recent review by Abu-Ghosh et al. (2021) have illustrated the suitable techniques of combining the stressed conditions for growth with enzymatic disruption process to extract the bioactive components/biostimulants from microalgae. Few reviews by Colla and Rouphael, (2020) and Kapoor et al. (2021) have provided the much required insights into the potential of microalgal biostimulants to revolutionize the global food production systems. Also, a recent report on the trends of research related to microalgae in Europe by Rumin et al. (2020) have identified microalgal biostimulants as an emerging concept with the scope for further research. None of the reviews done so far have extensively elaborated on technical and economic criticalities necessary for commercialization. The reviews by Abu-Ghosh et al. (2021) have briefly presented the markets and costs of the algal biostimulants with no discussions about the market credibility and policies. Kapoor et al. (2021) have made stupendous attempt to cover the market credibility (mainly restricted to Europe) and even provided insights into the resource recovery options to increase its commercialization aspects. However, systematic details over the mechanism of these biostimulants

on the metabolism linked with plant growth and also, the environmental and economic constraints linked with the production of microalgal biostimulants were not discussed. Since, the commercialization potential is often hindered by the high costs and emissions during the upstream and downstream process of extraction of these high value bio-based compounds from microalgae (Tang et al., 2020), it is necessary to understand the existing markets, revisit the ecological and technical constraints, further redesign the microalgal bioprocess based on the principles of resource recovery to attain maximum benefit from the existing algal biorefinery concepts.

Contrary to the previous reviews published with similar background, summarizing only the plant growth potential of microalgal biostimulants, the objective of the present review is to comprehensively put-forth the circular bioeconomy aspects to promote its market credibility. To fill the existing knowledge gaps, the study describes the existing microalgae (single species/consortium) with essential secondary metabolites that holds significant potential for biostimulant application and elucidates their action mechanism over plant metabolism, with insights into the intracellular pathways involved. The present study was restricted to evaluate the biostimulatory role of only eukaryotic microalgae excluding prokaryotic cyanobacteria because of its associated controversial phytotoxic effects in case of extraction of whole cellular level metabolites. The existing market trends and opportunities for the high value microalgal biostimulants, including the associated country-wise policies and regulations for market entry and colonization are deliberated. The real-time feasibility of market expansion of microalgal biostimulants hindered by the enviro-economic constraints are described considering decisions from the techno-economic and life-cycle assessment studies. Furthermore, perspectives on improvising the supply chain via integration of resource recovery from waste streams through robust strains identified by high throughput phenotyping in a systematic algal biorefinery to expand the market are described. The review is expected to guide the researchers, industrialists and policy makers by bringing out the clear picture on the present status of microalgal biostimulants not only in terms of basic lab/field scale research but also the challenges linked with its real-time market potential. It is expected to act as a guide to inculcate sustainable practices providing future directions to commercialize the high value microalgal biostimulants with the aim of achieving viability and social equity in circular bioeconomy.

2. Specific microalgae and consortium as the source of bioactive metabolites

Biostimulants usage from eukaryotic microalgae is still at its nascent stage. Several microalgal species are yet to be fully explored for their bioactive potential for promoting plant growth and development. Very few microalgae like *Chlorella* sp., *Scenedesmus* sp., *Dunaliella* sp., *Nannochloropsis* sp., and *Haematococcus* sp. are mostly frequently utilized as the source of biostimulants (Kapoor et al., 2018). The lack of knowledge on the detailed metabolite content of most eukaryotic microalgae have restricted their potential utilization for biostimulatory action. Thus, in an attempt to provide better insights, Table 1 summarizes the microalgae (single / mixed consortium) along with the concentration of the high value metabolites present, that could be utilized as biostimulants. These compounds are usually required in small concentration to improve the performance and growth quality of plants. The study by Ricci et al. (2019), reported that in case of microbial biostimulants, it might not be practically possible to establish the requisite dosage, as several influencing factors governs the performance of biostimulants during field scale application. It is noteworthy to mention that the minimum requisite dosage or concentration for any biostimulant to perform depends on the type of plants as well as the mode of application (seed pre-treatment and foliar spray). Each plant has its own requirement for specific biostimulants with optimum concentration that are to be determined based on trials.

Table 1

List of microalgal species, their bioactive/metabolite components and physiological actions.

Microalgae/ consortium	Metabolite/ Bioactive component	Quantity	Potential action	References
<i>Scenedesmus armatus</i> , <i>Chlorella minutissima</i> , <i>Chlorella pyrenoidosa</i> <i>Chlorella vulgaris</i>	Auxin (IAA and IAM)	IAA – 0.5 to 71.49 nmol/ g DW, IAM – 0.18 to 99.83 nmol/g DW	Biosynthesis of pigments	Lu and Xu, (2015)
<i>Chlamydomonas reinhardtii</i> , <i>Dunaliella</i> sp., <i>Chlorella minutissima</i> <i>Haematococcus pluvialis</i>	Absciscic acid	3 to 34 nmol/g DW	Systemic stress tolerance	Lu and Xu, (2015)
<i>Chlorella minutissima</i> , <i>Chlorella vulgaris</i> , <i>Klebsormidium flaccidum</i>	Cytokinins	0.29 to 21.40 nmol/g DW	Cytokinesis (cell division), cell growth, accumulation of photosynthetic pigments	Stirk et al., (2013a)
<i>Chlorella</i> sp., <i>Chlamydomonas reinhardtii</i> , <i>Raphidocelis subcapitata</i>	Gibberellins	0.86 to 13.5 nmol/g DW	Growth and metabolism	Stirk et al., (2013b)
<i>Chlorella pyrenoidosa</i>	Ethylene	–	Growth and metabolism	Lu and Xu, (2015)
<i>Tetraselmis</i> sp., <i>Dunaliella salina</i>	Pyridine –3- carboxamide	–	Primary precursor of (NAD +) in ATP synthesis and the sole substrate of poly-ADP-ribose polymerase-1	Mutale-Joan et al., (2020)
<i>Tetraselmis</i> sp., <i>Dunaliella salina</i>	Phytol (alcohol terpenes)	0.02 to 0.08 g/g DW	Constituent of chlorophyll, biosynthesis of tocopherols, protection of Photosystem II from environmental stress, enhanced lipid biosynthesis	Mutale-Joan et al., (2020)
<i>Chlorella vulgaris</i>	Polysaccharides	174.46 to 543.4 mg/g DW	Structural support and energy storage, antioxidant activity	Elarroussia et al., (2016);El-Naggar et al., (2020)
<i>Chlorella</i> sp., <i>Anikistrodesmus</i> sp., <i>Dunaliella salina</i>	Carotenoids	4.85 to 7.2 mg/g DW	Associated with capturing light for photosynthesis and protection of from high incident light via non- photochemical quenching (photoprotection)	Pisal & Lele, (2005)
<i>Chlorella</i> sp., <i>Chlamydomonas reinhardtii</i> , <i>Klebsormidium flaccidum</i>	Brassinosteroids	0.023 to 2.03 nmol/g DW	Resistance against heat stress, enhances antioxidant activities and carboxylation	Stirk et al., (2013b)
<i>Chlorella vulgaris</i> <i>Scenedesmus obliquus</i>	Proteins and amino acids	0.18 to 0.46 g/g of DW	Precursors for phytohormones and polyamines in embryogenesis and organogenesis with osmotic stress protection	Becker, (2007)
<i>Chlorella vulgaris</i> , <i>Nannochloropsis</i> sp., <i>Tetraselmis</i> sp.	Antioxidants	3.3 to 90 µmol trolox eq./g DW	Required for protection from ROS and free radical scavenging	Goiris et al., (2012)

IAA – Indole-3-Acetic Acid; IAM – Indole-3-Acetamide; DW – Dry weight; ROS – Reactive oxygen species

Microalgae are a rich harbour for the presence of significant quantities of phytohormones (Indole acetic acid; Absciscic acid; Cytokinin; Ethylene; Gibberellins), polyamines, antioxidant compounds, vitamins, along with polysaccharides and amino acids that have synergistic influence on overall metabolism of plants (Kapoor et al., 2018). Unlike cyanobacteria which possess cyanotoxins that might have phytotoxic influence on plant growth as reported by Bouaïcha & Corbel, (2016), microalgal biostimulants are considered safe with no phytotoxic compounds (Ronga et al., 2019). Plaza et al. (2018) reported that extracts of *Scenedesmus* sp. with IAA and absciscic acid enhanced the root growth of *Petunia hybrida*. Oancea et al. (2013) has reported the alleviation of water stress in tomato plants due to exogenous application of cytokinin from *Nannochloropsis* sp. Lu et al. (2014) reported that the genes regulating cytokinin and absciscic acid biosynthesis are upregulated during nutrient stress, while the former stimulates cell cycle progression, the later provides stress tolerance. Van de poel et al. (2016) through transcriptome analysis found a large set of genes regulating ethylene synthesis in *Spirogyra platensis* controlling photosynthesis, chlorophyll synthesis, plant cell remodelling response to abiotic stress. Brassinosteroids (BRs) (brassinolide and castasterone) is a common phytohormone detected in microalgal strains, which have been reported to provide resistance against heat stress, and enhances the antioxidant activities as well as the carboxylation, thereby, the overall plant growth (Kapoor et al. 2018). Synergistic action of BRs with ethylene provided hypocotyl elongation, and in combination with absciscic acid generated drought resistance. Apart from BRs, absciscic acid is also abundantly present in *C. vulgaris*, *S. quadricauda*, *C. sorokiniana*, *N. oceanica*, *C. reinhardtii*, *D. salina* and *H. pluvialis* (Lu et al., 2014; Pan et al., 2019). Salicylic acid and Jasmonic acid found in almost all microalgae aids in defence responses of the plants against herbivorous insects, necrotrophic and biotrophic pathogens respectively. Plaza et al. (2018) reported significant presence of these acids in *Scenedesmus* sp.. Jasmonic acid and pathways for the synthesis of linoleic acid (precursor of jasmonic acid) were also detected in *Chlorella* sp. (Tarakhovskaya et al., 2007). It was

proposed that the concentration of linoleic acid declines under salt stress, thus supplementing these extracts from microalgal species could successfully alleviate salt stress.

Polyamines like spermine present in microalgae regulate stress responses in plants. Microalgal spermine extracts applied to lettuce increased its spermine content by 64% in plant leaves making it more robust to environmental stress (Mógor et al., 2018). Microalgal extracts with higher amino-acids or protein hydrolysates promote the overall nutrient uptake, assimilation and metabolism in plants increasing resistance of plants to heat, cold, oxidative damage, draught and increased salinity (Bulgari et al., 2019). Microalgae from *Chlorella* sp., having more than 40% amino acid content mostly dominated by aspartic acid, glutamic acid, leucine, arginine, isoleucine are regarded ideal for biostimulants (Hempel et al., 2012). Plaza et al. (2018) reported higher fresh and dry weight of *Petunia* treated with 10 g/L protein hydrolysate (enzymatically treated extracts) of *Scenedesmus* sp., due to increase in growth and metabolism. Humic substances reportedly associated to improve plant growth have yet not been confirmed from microalgal source, though the study by Heilmann et al., (2011) have reported the presence of an insoluble brown precipitate in organic solvent after acidification of *C. reinhardtii* extracts.

Most microalgae contain polysaccharides made up of glucose, galactose, mannose, xylose, arabinose in distinct proportions linked by glycosidic bonds which aid in overall plant growth, promoting resistance against stress factors (Rachidi et al., 2020). Heteropolymer of β-(1,3)-glucan was detected in *C. vulgaris* by Chanda et al. (2019). Arroussi et al. (2018) reported maintenance of potassium: sodium ratio during salt stress, thereby promoting healthy growth via application of exopolysaccharides from *D. salina*. Polysaccharides from *C. vulgaris*, *C. reinhardtii*, *C. sorokiniana* and *D. salina* have been reported to inhibit ROS toxicity (Farid et al., 2019). A recent study by Rachidi et al. (2020) also reported the presence of significant amount of polysaccharides containing neutral sugars, sulfate groups and uronic acids in *D. salina*, and *Porphyridium* sp., crude extracts, which could have significant

biostimulatory action.

B. braunii, *N. oleoabundans*, *H. pluvialis*, *C. vulgaris*, *P. tricornutum* and *Isochrysis* sp., have significant antioxidant capacities due to high chlorophyll, carotenoids, phenolics, vitamin C and E (Shebis et al., 2013). Andrade et al. (2018) reported that *Chlorella* sp., contain phloroglucinol, apigenin, ferulic acid and p-coumaric acid. *C. calcitrans*, *I. galbana*, *S. costatum*, *O. sinensis* and *P. tricornutum* contain significant amounts of phenolic compounds like fucoxanthin and gallic acid (Foo et al., 2017). These compounds provide better resistance in plants during environmental stress. However, their activity is much less explored. Certain microalgae however, depending on the growth conditions might possess minimal concentration of allelochemicals like cyclic peptides and volatile organic compounds which could have antibacterial, antifungal and anti-insecticidal / pesticidal activity (Kapoor et al., 2021). The study by Bileva, (2013) showed that the *C. vulgaris* extracts applied over grape seedlings provided photo-protective effects. Although the concentration and exact mechanism of action of these compounds are still undiscovered, often maintenance of optimal growth conditions, keep the intracellular concentration of these toxic chemicals in microalgae below the threshold range preventing negative effects during the whole-cellular extraction. Microalgae also have appreciable quantity of vitamins (B and C), fatty acids and terpenoids that are expected to have antimicrobial, antioxidant activities and could be used as bio-pesticide for post-harvest disease management (Kapoor et al., 2021).

3. Microalgal biostimulants: Action mechanisms and applications

The application of microalgae as a plant growth stimulant usually involves the extraction of bioactive compounds from the whole biomass. The bioactive components from microalgae can be extracted following different physical pre-treatment processes (autoclaving; high pressure extraction; microwave or ultrasonic treatment) (Garcia-Gonzalez and Sommerfeld, 2016) or via the use of chemicals like acids/alkali (Chiaiese et al., 2018), green solvents under supercritical or subcritical mode and even enzymatic methods (Plaza et al., 2018). The physical extraction procedures are energy intensive, while the solvent based and enzymatic extraction process are costly, and unattractive during scale-up. Often the physical methods are co-combined with the chemical cell pre-treatment to increase the yield (Kapoor et al., 2018). Among all these techniques,

supercritical CO₂ extraction is more economically feasible as inexpensive CO₂ is the solvent and involves mild conditions of temperature (~30 °C) at high pressure (~35 MPa) (Michalak et al., 2017). Moreover, extracted compounds can be easily separated through CO₂ elimination by reducing the pressure.

These bioactive compounds often provide an improved systemic resistance to the overall plant along with increased nutrient utilization and photosynthetic efficiency against different biotic and abiotic stress conditions. Some of the inherent putative response mechanisms observed in plants (Fig. 1) are listed below:

- Plant growth responses through biochemical changes in the photosynthetic pigments, higher yield and quality with delayed senescence.
- Biotic stresses response include resistance to pathogens of bacterial, fungal and viral organisms and even to insect, pests and weeds.
- Abiotic stresses response consists of resistance towards drought and salt contents, and chilling and freezing tolerance.
- Whole plant responses towards biostimulants include reactive oxygen species (ROS) scavenging, membrane stability, osmo-protection and ion homeostasis.

The above-mentioned responses are mainly attributed to the cascade of metabolic variations generated intracellularly inside the plant cell following elicitor perception. This process causes the upregulation of a series of genes involved in primary plant metabolic responses, resulting in increased activity of rhizobacteria and other essential microbiome modulating the root architecture, thereby the nutrient uptake efficiency (Ronga et al., 2019). The intracellular molecular mechanism starting from the elicitor perception to the requisite metabolite generation has not yet been described in detail by any reviews so far. Since, the chemical nature of the biostimulants extracted from microalgae is similar to macroalgae, it is expected to initiate similar signalling pathways inside the plant cell as described in the study by Ali et al. (2021). As illustrated in Fig. 2, elicitor perception often results in a series of metabolic response thereby, causing reversible phosphorylation and dephosphorylation of the proteins present in the plasma membrane and the cytosol. This in turn increases the calcium (Ca²⁺) ion concentration, thereby causing an efflux of chloride (Cl⁻) and potassium (K⁺) ions with increased influx of protons (H⁺). Eventually, the cytoplasm becomes

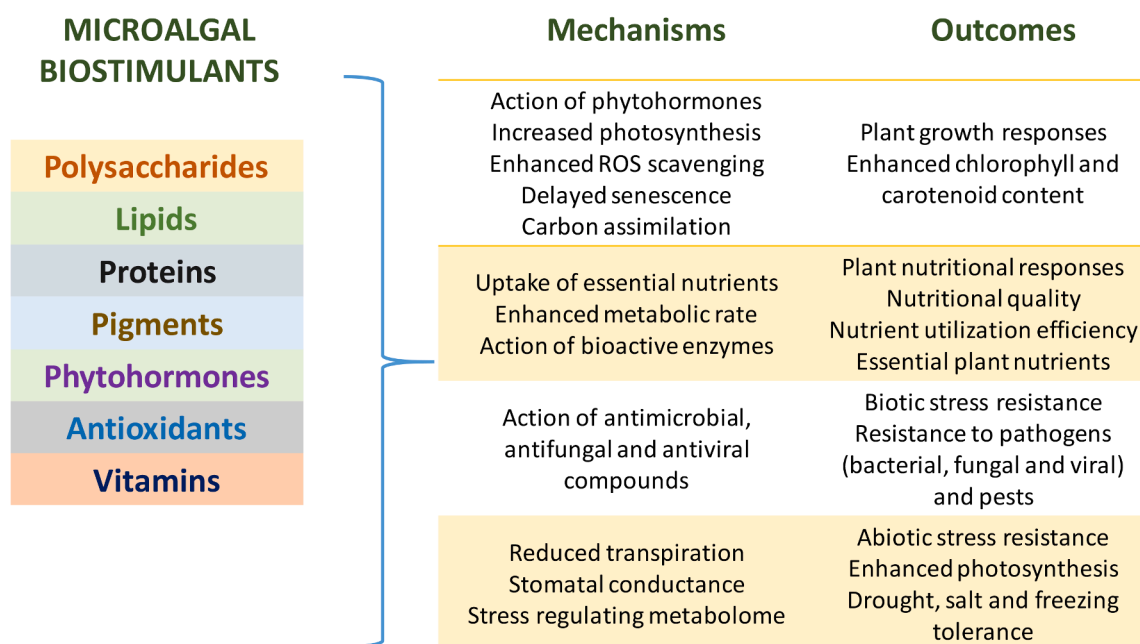


Fig. 1. Action of microalgal biostimulants with the mechanisms and possible outcomes.

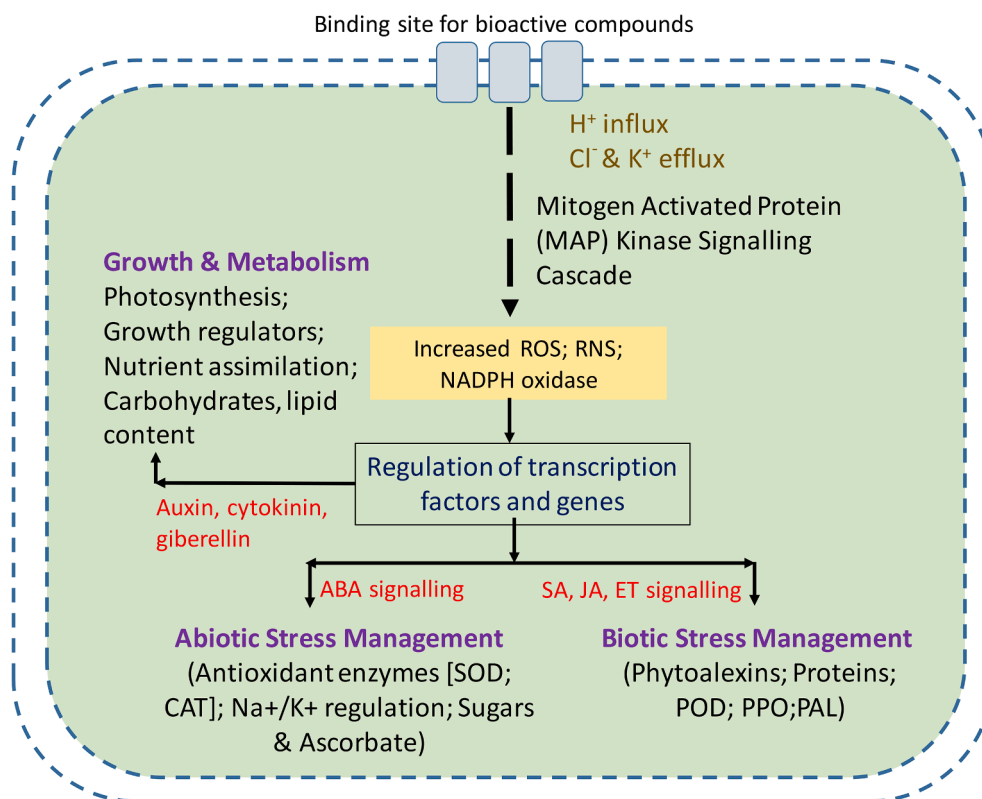


Fig. 2. Cellular pathways and actions of bioactive compounds influencing the systemic plant resistance.

acidified while the plasma membrane is alkaline. This results in rapid generation of ROS and reactive nitrogen species (RNS) which initiates a cascade of reactions involving phytohormones resulting in increased growth and metabolism providing tolerance towards abiotic and biotic stress conditions.

The amount and concentration of bioactive metabolites in microalgae is dependant over the algal species and the growth conditions (Kapoor et al., 2021). Instead of extracting bioactive metabolites individually, these compounds always act together better, thus must be obtained altogether using a systematic approach. The microalgal extracts can be applied either as a seed pre-treatment agent or via foliar spray. In certain cases, these chemical compounds are extracellularly secreted, thus whole algal wet biomass can also be directly applied into

the roots of plants in a hydroponic co-cultures (Supraja et al., 2020a). Table 2 illustrates the microalgal species, their formulations, method of extraction involved, the requisite dosage and application duration of the biostimulants. Many microalgae listed in the table still remain unexplored for their biostimulatory potential, thus directing the need to employ further research to fill out the knowledge gap.

The liquid extracts rich in bioactive metabolites can be applied at any time during the growth phase; even before (specially having compounds like proline and other amino acids) or during the onset of stress conditions (in case of bioactive compounds rich in phytohormones) to the plant (Drobek et al., 2019). Being easily available in an assimilatory form, these compounds act very fast, but their efficiency might not last longer, thus facilitating more frequent dosages. It is imperative to

Table 2

Formulations, bioactive element concentration and application dosage of microalgal biostimulants.

Formulation	Microalgae	Biostimulant concentration & Extraction method	Application dosage or Duration	References
Foliar spray	<i>Acutodesmus dimorphus</i>	1, 10, 25, 50, 75 and 100% extracted with microfluidizer at flow rate of 450 ml min ⁻¹ and 172 mPa	Sprayed at the time of transplant and after 4 weeks	Garcia-Gonzalez and Sommerfeld, (2016)
Foliar spray	<i>Scenedesmus</i> sp.	0, 0.1 and 0.2% of algal extracts	60, 75 and 90 days after planting	El-Khair et al., (2010)
Foliar spray	<i>Scenedesmus</i> sp.	10 g/L of each microalgae subjected to high pressure homogenization and enzymatic hydrolysis with proteases	0, 14, 28, 35, and 42 days after transplanting	Plaza et al., (2018)
Seed primer	<i>Acutodesmus dimorphus</i>	1, 10, 25, 50, 75 and 100% extracted with microfluidizer at flow rate of 450 ml min ⁻¹ and 172 mPa	–	Garcia-Gonzalez and Sommerfeld, (2016)
Seed primer	<i>Chlorella vulgaris</i> , <i>Isochrysis</i> sp., <i>Nannochloropsis</i> sp., <i>Scenedesmus</i> sp. and <i>Tetraselmis</i> sp.	50 mg dried biomass in 1 L water lyophilized at –20 °C and pressure of 0.320 mbar	4 ml of extract	Ronga et al., (2019)
Seed primer	<i>Chlorella vulgaris</i>	Supernatant after biomass removal	–	Faheed and Fattah, (2008)
Seed primer	<i>Chlorella vulgaris</i> , <i>Chlorella protothecoides</i> , <i>Synechocystis</i> sp., <i>Tetraselmis obliquus</i> ,	0.5 g/L of each culture	5 days in dark and 5 days in sunlight	Ferreira et al., (2021)

understand their application time and dosage to further rationalize their utilization with much lower investments during the microalgal biostimulant production stage. Studies by Supraja et al. (2020b) showed that efficacy of these compounds is a function of their concentration, where after a certain threshold level, the effect of the biostimulants becomes almost constant. The optimal dosage and the application rate is dependent on the strength (~referring to the presence of different bioactive elements) of the microalgal extracts (Arnaud, 2016). The study by Supraja et al. (2020b) reported that 40% and 60% of the whole cellular extracts of mixed microalgal consortium showed the maximal seed pre-treatment and foliar spray efficiency, and the effect becomes almost constant with further increase in microalgal concentration. Similar to the above study, Garcia-Gonzalez and Sommerfeld (2016) reported that 50–75% cellular extracts of *Acutodesmus* sp., showed maximal efficiency during foliar spray and seed priming agent for tomato plants. Thus, the extractability of the physical or chemical process employed play an essential role in determining the concentration of bioactive compounds in the extractant medium. Nevertheless, it is essential to understand the key steps of i). efficiency / extractability of the physiochemical process for cell disruption to obtain the intracellular metabolites along with the ii). application time, dosage and frequency to design downstream processes which will be energy saving as well as cost efficient and eco-friendly, promoting sustainable utilization of these components.

4. Market trends, opportunities and criticalities of microalgal biostimulants

4.1. Market size, share and opportunities

Biostimulants are deemed as a popular and eco-friendly substitute of chemical fertilizers for sustainable agricultural development. Based on the biostimulant market forecast reports (2019), the global biostimulants market is expected to grow in terms of time value of money at cumulative annual growth rate (CAGR) of 13.4% from 2019 to 4.47 million dollar in 2025. Volumetrically, the biostimulant market is predicted to reach 446,651 metric tons by 2025 with a CAGR of 12.4% (Meticulous Research, 2021). This projected growth rate is higher than the growth rate of 1.3–1.8% annually reported for inorganic chemical fertilizers. Biostimulants targeted for foliar spray occupy a higher share of global market in terms of the modes of application followed by those targeted for roots and seed pre-treatment. It is noteworthy to mention that the biostimulants for seed pre-treatment occupies a higher share of revenues.

Biostimulant market is mainly segmented based on the i). Nature of product i.e. acidic and extract based ii). Crop type (cereals and grains; fruits and vegetables, ornamentals and turfs) and iii). Application (foliar spray, seed treatment, soil treatment). Humic substances (acidic extracts) dominate the biostimulant markets followed by seaweed extracts. Presently, the share of macroalgae and humic substances is almost 70% of the total market share of global biostimulants (Transparency Market Research, 2019). Biostimulants based on use are mostly developed for cereals and grain based crops, followed by its application in vegetables and fruits, and then for ornamental and turf plants. Country wise, the markets for biostimulants are more diversified in Europe, followed by North America, Asia-Pacific and, Latin America, Middle East and African (LAMEA) countries (Brazil, Argentina, UAE, Saudi Arabia, South Africa, Nigeria etc.). According to Rumin et al. (2020) microalgal biostimulants are one of the emerging algae based product markets in Europe. As per the European Biostimulant Industry Council (EBIC), the continent occupies almost 50% of the total global biostimulant markets, mostly consisting of protein hydrolysates, humic acid substances and macroalgal extracts. The market is expected to grow at a CAGR of 10–12%, reaching a net worth of 1.5–2 million US \$ by the year 2022. According to Arnaud, (2016), European markets occupy 30% of the total global revenues from worldwide biostimulant markets, which are utilized in

over 8.5 million hectares of total European land (EBIC Report, 2016).

Both microalgal and macroalgal extracts occupy 40% of the total share of biostimulant markets according to the reports of European commission, (2016) (EBIC Report, 2016). Researchers and biostimulant industries have started exploring microalgae based products as these phototrophs cultivated under optimized conditions in outdoor ponds provide a much consistent biochemical composition and other metabolites for crop application. Presently, the global value of microalgal biostimulants amounts to 2.5 billion Euros (Barsanti and Gualtieri, 2018). The cost of biostimulants varies between 10 and 80 Euros, further, depending on the crop type, application frequency and dosage, the treatment price varies between 100 and 600 Euros per hectare (Arnaud, 2016). Over years, many microalgal biostimulant companies have been established throughout the globe as listed out in Table 3. Most companies deal with the sales of microalgal biostimulants as foliar spray agent and are located in Spain and Turkey. These companies often face tremendous competition from the synthetic chemical counter parts due to the higher time and costs involved during the production until the market entry period (El-Boukhari et al., 2020). Biostimulants in Indian scenario had a worth of 71.23 million US dollar in 2017 and is expected to witness a CAGR of 16.49% in the forecast period up to the year 2024, reaching a total value of 180.95 million US dollar (Size et al., 2019). Algae based biostimulant companies in India are extremely limited, with most industries utilizing seaweed biomass as biofertilizer. In recent years few companies, like Soley Biotech, Hindustan Bioenergy Limited have started exploring microalgal strains from *Chlorella* sp., *Scenedesmus* sp., and *Nannochloropsis* sp., as the source of bioactive metabolites for biostimulant application.

4.2. Policies and regulations

The demand for healthy, safe food and crops have increased over the years, however, compared to other organic alternatives, the algal biostimulant markets have not expanded to its full extent. The reasons being the lack of market credibility and longer market time window (usually 5 years). Further, the absence of sufficient reproducible results at laboratory that could be translated to field scale, co-combined with stringent regulations and policies, constraints their commercialization (Arnaud, 2016). A suitable regulatory framework is expected to consider all the economic and environmental considerations to promote sustainable utilization of biostimulants (du Jardin, 2015).

The present regulatory frameworks vary based on different countries and locations, and do not incorporate any specific or standardized procedures that are globally consistent. European Union (EU) is the first country to establish regulatory frameworks for biostimulants as separate entity from that of biofertilizers. EU has two governing bodies i.e., European Crop Protection Agency which deals with the pesticide control issues and Bio-based Industries Consortium (a public private partnership organization) which facilitates the development of sustainable bio-based industries in Europe (El-Boukhari et al., 2020). Three main regulations governing the use of microalgal biostimulants currently followed are, i). EC Regulation No. 2003 pertaining to the use of fertilizers; ii). EC Directive No. 2009 concerning sustainable use of pesticides and iii). EC Regulation No. 1107/2009 for products concerning plant protection (Dmytryk and Chojnacka, 2018; Kapoore et al., 2021). Since, “biostimulants” by definition do not supply any nutrients and is utilized to promote the plant growth and metabolism boosting its response to biotic and abiotic stress, these high value compounds fall under the legislation of EC 1107/2009 of plant protection products (du Jardin, 2012). Further, these bioactive elements can only be utilized based on their actions over plants at field scale. In relation to the above-mentioned aspects, it is noteworthy to mention that microalgal biostimulants often face challenges as the number of field trials are limited and also the results obtained varies with geographical locations. These value added chemicals follow the same legislation as that of any non-genetically modified microbial products to process and extract the bioactive

Table 3

Major microalgal biostimulant companies and their product features across different countries.

Company	Brand name	Microalgae	Composition	Product/dosage	Average price	Revenue	References
AlgaEnergy (Spain)	Agrialgae®	Mixotrophic microalgal culture	Proteins, carbohydrates, phytohormones, polysaccharides, carotenoids, pigments	Foliar(2 – 3 ml/L)	25 € per litre	6,000,000 \$	AlgaEnergy. Agrialgae® Available online: https://algaenergy-intl.com/new-biostimulants/ (accessed on 24 May, 2021)
Agroplasma SI (Spain)	Ferticell® Universal™	Microalgal extract	Amino acids and plant beneficial compounds	Foliar(2–5 ml/L)	15 € per litre	2,000,000 \$	(SI, 2021)
MCT Tarim Ltd. Sti (Turkey)	EMEK	<i>Chlorella</i> sp.	Polysaccharides and trace elements	Foliar(2.5 ml/L)	–	–	MCT Tarim, (2021)
Natur Agro (Hungary)	Natur Plasma® and Natur Vita®	<i>Chlorella vulgaris</i>	Phytohormones, amino acids, nutrients	Foliar(direct use)	3.77 € per litre	2,646,536 €	(NaturAgro, 2021)
Allgrow AB (Sweden)	Allgrow®	<i>Chlorella</i> sp.	Phytohormones mainly cytokinins and auxins	Foliar(0.5 – 5 ml/L)	–	–	(Comp, 2021)
Heliae development LLC (USA)	PhycoTerra® ST	Mixotrophic microalgal cultures	Whole microalgal biomass	Seed treatment (3% v/v)	125–250 \$ per acre	20,000,000 \$	Heliae development LLC. Phycoterra®. Available online: https://phycoterra.com/products/seed-treatment/ (accessed on 24 May, 2021)
Mikroalg Food and Agriculture Industries (Turkey)	Terradoc®	<i>Chlorella</i> sp.	Whole microalgal biomass (approx. 1×10^7 cells per ml)	–	–	3,000,000 \$	(Inc, 2021)

compounds via physical and chemical processing technologies, which could be sold in market with the tag of biostimulants/plant protective compounds. However, constraints do exist while obtaining organic certifications (EU Regulation 2018/848) as the regulation restricts the use of algal biomass grown in wastewater / anaerobic digestate / manure as a part of the feed chain even when there are no phytotoxic and eco-toxic effects (Commission Reports, 2018a,b). These circumstances require the involvement of European Food Safety Corporation for suggestions to authorize and deliver the proposed product into markets. In United States (US), the US Biostimulant Coalition and the Biological Product Industry Alliance are working together to develop stringent regulation to differentiate these biologically extracted compounds from other categories. These two organizations along with the US Environmental Protection Agency are working to formulate clear and consistent rules all over US to promote market credibility, facilitating easier market entry of these products (du Jardin, 2015).

The algal biostimulant is a relatively new product with no separate recognition or specific rules for recognition in Asia-Pacific countries especially in China and Japan, and these products are often marketed under the broad category of organic fertilizer (Biological Industry Product Alliance Report, 2018). The Government of India, with Indian Chamber of Food and Agriculture (ICFA) aims to formulate regulatory bodies to facilitate safe and eco-friendly entry of these biochemicals, with specific focus on labelling their composition having toxic pesticide components or heavy metals at lower limits (Biological Industry Product Alliance Report, 2018). The Federation of Indian Chambers of Commerce and Industry (FICCI) categorize biostimulants as speciality products permitting market entry abiding to the rules and regulations as proposed for macronutrients and biofertilizers. Since, the biostimulant market space in India is mostly dominated by the small and medium enterprises, government funding in research and development can definitely drive innovations, while more stringent norms related to bio-efficacy and field trials often lead to small ventures moving out of business. Thus, establishment of a uniform policy and decision making process with standardized portfolios can surely help in promoting advancement of these sectors.

5. Enviro-techno-economic constraints for commercialization of microalgal biostimulants

Even though there has been a tremendous research over years on algal biofuels, the exploration of microalgae as a “fuel only option” is not

economically viable due to inappropriate techno-economic performances. Undoubtedly many techno-economic feasibility and life-cycle impact evaluation studies have been done for microalgae based bio-energy production (Batan et al., 2016; Rajesh Banu et al., 2020) but, the economic feasibility assessment dedicatedly directed towards biostimulants production are extremely limited. However, since the basic process flow for scaling up algal production remains mostly same including the cultivation; harvesting and extraction, the conclusions retrieved by comprehensive studies done by the researchers on different algal biorefinery product conversion routes could surely help to identify the major bottlenecks for the commercialization of microalgal biostimulants.

Several technical constraints could be possibly projected for the commercialization of microalgal biostimulants starting from the process of cultivation until the final formulation and product packaging. Mass cultivation of microalgae occupies maximum share of the total process costs (Espada et al., 2020). Although the volumetric productivity of microalgal biomass through photobioreactors is higher than open ponds, both these systems have got industrial relevance for high quality biomass production (Huesemann & Benemann, 2019). It is easier to construct, operate and maintain open raceway ponds compared to the closed photobioreactors (PBRs), though the later provides higher biomass productivity and potency due to better controllability (Thomassen et al., 2016). Cost of algal biomass varies from 0.5 to 6 \$/kg depending on the open or closed photobioreactor designs (Kumar and Singh, 2019). Raceway ponds are often prone to contamination compared to the closed photobioreactors. However, the repeated sub-culture of microalgae using cheaper waste resources like wastewater or anaerobic digestate can be used to avert the issues. Thus, it could be postulated that open ponds with a robust microalgal strain, well-acclimatized with repeated subculture could act as suitable method for maximum production of biostimulants. Contrary to these reports, the study by Spruijt et al. (2015) projected a contrary that the biomass production cost is not much influenced by the reactor type, but is more sensitive to the species type, its growth rate, biomass productivity and the extent and source of carbon dioxide (CO₂) supplied. Further, the growth phase is also associated with higher water footprints and also the land use compels additional costs and environmental impacts (Thomassen et al., 2016). Most commercial scale plants utilize synthetic fertilizers with freshwater to grow microalgae. Apart from the higher costs associated with the utilization of synthetic fertilizers in bulk, high consumption of freshwater is also expected to increase the unit price of

freshwater (Farooq et al., 2015). The study by Zaimes and Khanna, (2013) reported that the water demands of the process are sensitive to the geographical regions and might be one of the serious constraining factors influencing the economics of algal cultivation. A detailed variation of algal biomass costs as a function of the cultivation process has been provided by Wijffels and Barbosa (2010) and Norsker et al. (2011).

The next major steps adding to the unfeasible process economics is the harvesting and extraction. The use of chemical flocculants and solvents involved during harvesting and extraction respectively along with the costly equipment increases the energy consumption and makes the entire downstream process cost intensive (Thomassen et al., 2016). The dehydration and extraction of bioactive compounds in most microalgae contributes to 20–30% and 40–50% of the total process energy requirements respectively (Dasan et al., 2019). The harvesting process in fact generates a huge amount of residual water, which must be treated before disposal, incurring additional process costs. Techno-economic feasibility studies along with the expenditure and the projected revenues for obtaining bioactive metabolites have been summarized in Table 4. It is noteworthy to mention that in most cases, the exploration of multiple products often brings down the payback time of a project providing higher rate of returns, compared to that of obtaining a single product. Further, most studies do not take into account the purification and packaging of the bioactive compounds, which is also expected to raise the overall product cost. Thus, more studies considering the unit step involving product packaging in future might provide a much more realistic biostimulant cost estimate.

In addition to economic feasibility, to mitigate the negative impacts over the environment, it is essential to utilize the life-cycle assessment (LCA) tools to access the ecological indicators and environmental impacts of each of the unit process. Table 5 presents the impact assessment methods utilized for different unit processes starting from cultivation until downstream processing and lists out the critical hotspots associated in the cradle to gate analysis. Most studies listed in the above-mentioned table identifies cultivation, followed by extraction as the major steps contributing to highest environmental impacts. On similar grounds, Zaimes and Khanna, (2013) reported that the energy and the environmental impacts of cultivation and harvesting of algal biomass contributes to the maximum share of total life-cycle impacts and is also location dependent. The study by Pérez-López et al. (2014) identified the inoculation and mass culture of microalgae as the major hotspot for environmental impacts, which could range from 73% to 97% depending on the category of impacts. Among the impact categories, GHG emissions

via the use of electricity during the operation of PBRs, associated pumps and supply of CO₂ enriched air constituted 51%, 7% and 0.3% of the total emissions, respectively. This is because the electricity utilized is usually being considered from local grid which uses fossil fuels as the energy source. Also, the production of sodium nitrate (N source commonly utilized during cultivation) contributes to highest cumulative energy demands, eutrophication potential along with 93% and 100% nitrous (NO_x) and ammonium emissions, respectively. The next important unit process contributing to a major share of environmental impacts is the extraction, which consumes 42% of the total electricity requirements. The solvent recovery steps add to a major share (99%) of electricity requirements during the extraction step, apart from the ecological impacts associated with the utilization of chemical solvents. Use of bio-based solvents (prepared from renewable resources) like ethyl acetate /lactate or a combination of 2-methylhydrotetrafurane along with water for cold-compressed extraction of bioactive compounds as demonstrated in the study by Damergi et al. (2017) and Derwenskus et al. (2019) could act as a cleaner alternative. But the cost-impacts of the process might not be attractive enough. A recent study on LCA on cultivation of *P. tricornutum* to produce bioactive compounds rich in antioxidant and anti-inflammatory properties by Porcelli et al. (2020) showed cultivation (~50%; mostly from the synthetic nutrients production phase) and freeze-drying (~40%) as the most critical stages contributing to environmental and ecological impacts. The study proposed that the use of a renewable energy mix and waste CO₂ produced during upgrading of biogas rather than a synthetic source could bring down these impacts.

6. Perspectives on expanding the microalgal biostimulants commercialization potential

6.1. Establishing a sustainable circular economy via biorefinery approach

It is a well evident fact that most of the microalgal bioactive compounds fail to reach the market due to the unfeasible process economics. Thus, there is a prominent need to reduce the associated costs via utilization of waste resources, employing the principles of resource recovery and recycling (Kapoore et al., 2018). The two most commonly available waste resources that could be utilized as nutrient source promoting algae based circular bioeconomy are, i). Wastewater ii). Spent liquid from anaerobic biogas production termed as anaerobic digestate.

Different streams like the domestic wastewater, agricultural runoff,

Table 4
Economic feasibility assessment of microalgal bioactive metabolites.

Microalgae	Operational Conditions	Productivity/ Yield	CaPEX & OPEX	Total Revenues / ROI / Payback time	Inferences	References
<i>Chlorella</i>	Algae growth, harvesting, lipid extraction, conversion of proteins, and generation of pigments	10 tons of protein per day	CaPEX:138,127,000 \$/yrOPEX: 105,613,000 \$/yr	Total revenue: 173,906,000 \$/yr,ROI: 38.22%Payback time: 2.62 years	Only protein production will result in a payback period of 6.38 years3 products (protein, pigments and fatty acids) reduces the payback time to 2.62 years	AlMahri et al., 2019
<i>Scenedesmus obliquus</i>	Raceway pond cultivation, harvesting, extraction of proteins	6.4–8.34 MT/h153 MT protein per day	CaPEX:286,000,000 \$/yrOPEX: 146,000,000 \$/yr	Total revenue: 209,340,000 \$/yr	The economics of the process depends on algae protein content and productivity	Asiedu et al., (2018)
<i>Dunaliella salina</i>	Cultivation, centrifugation, drying, extraction, filtration, purification with solvent distillation	10.7 tons of β-carotene per year	CaPEX:18,270,000 €/yrOPEX:4,240,000 €/yr	Price of β-carotene is 920 €/kg	Cultivation is the costliest step followed by extraction and drying. Economics of the process are sensitive to the β-carotene content & it's extractability.	Espada et al., (2020)
<i>Nannochloropsis</i> sp.	Construction, cleaning, cultivation, harvesting, drying, extraction	50,917 kg protein rich biomass per year	CaPEX: 2.26 €/kg dry biomassOPEX: 7.35 €/kg dry biomass	ROI: 1.87%Payback time: 11 years	Most of the expenditure is due to infrastructure, maintenance and labour costs.	Schade & Meier, (2021)
<i>Chlorella vulgaris</i>	Algae cultivation, harvesting, extraction, isolation, purification	6.5 tonne of β-carotene per year	CaPEX:1,736,614 €/yrOPEX:504,710 €/yr	Total revenue: 4,270,500 €/yrprice of β-carotene:1370 €/kg	Construction and use of open ponds reduce the investment costs compared to photobioreactor	Özçimen et al., (2018)

CaPEX: Capital Expenditure; OPEX: Operating Expenditure; ROI; Rate of Interest

Table 5
Lifecycle and environmental impacts of microalgal bioactive metabolites.

Microalgae	System boundary conditions	Impact assessment methods	Outcome observed	References
<i>Tetraselmis suecica</i>	Sterilization, inoculation, cultivation and harvesting, extraction of α -tocopherol, chlorophyll and β -carotenoid using methanol and KOH	CML 2 baseline 2001 V2.04 method with SIMAPRO 7.3 softwareADP, AP, GWP, EUP, ODP, POFP, CED	The cultivation stage has the highest environmental impacts with 93% contribution margin followed by sterilization and inoculation stage (73%)	Pérez-López et al., (2014)
<i>Dunaliella salina</i>	Cultivation, centrifugation, drying, extraction, filtration, purification with solvent distillation	CML 2001 method with Gabi 6.0 softwareADP, EUP, GWP, HTP, ETP, FWEP, CED	Supercritical extraction step has lower energy consumption and environmental impacts due to toxicity of solvents involved	Espada et al., (2020)
<i>Chlorella vulgaris</i>	Cultivation, settling, centrifugation, oil extraction, carbohydrates and protein extraction	ReCiPe midpoint (E) method with SIMAPRO 7.3.3 software	Protein extraction had high energy demand and reduced environmental impacts with release of less carbon dioxide	Gnansounou and Kenthorai Raman (2016)
Microalgae (not specified)	Construction and cultivation in high- rate algal ponds, secondary settler, centrifugation, nutrient recovery, agricultural applications	ReCiPe midpoint method with SIMAPRO 8 softwareCCP, AP, ODP, EUP, POFP, HTP, ETP, PMFP, MDP, FDP	Cultivation systems established in warm temperature zones are more advantageous	Arashiro et al., (2018)

AP: Acidification Potential; ADP: Abiotic Depletion Potential; EUP: Eutrophication Potential; GWP: Global Warming Potential; HTP: Human Toxicity Potential; ETP: Terrestrial Eco-toxicity Potential; FWEP: Fresh Water Aquatic Eco-toxicity Potential; CED: Cumulative Energy Demand; ODP: Ozone Depletion Potential; POFP: Photochemical Oxidation Formation Potential; PMFP: Particulate Matter Formation Potential; MDP: Metal Depletion Potential; FDP: Fossil Depletion Potential; CCP: Climate Change Potential

piggery wastewater and industrial wastewater from tanneries, food processing, aquaculture are most commonly utilized to grow microalgae (Li et al., 2019). As microalgal cells are found to assimilate nitrogen and phosphorous compounds from wastewater, these nutrients can be removed, recovered for biomass production. This process of nutrient removal is safer, economical and cost-efficient compared to the conventional processes. Microalgae can remove 80–100% nitrogen and phosphorus along with 90% chemical oxygen demand (COD) (Shahid et al., 2020). The mechanisms involved in the major nutrient removal from municipal wastewater by microalgae are described in detail by Whitton et al. (2015). The nutrients present in wastewater can be suitably altered to influence the biomass yield and the metabolite content, thereby the applications and performance of algal biorefinery. The process is expected to make the cultivation step feasible not only in terms of reduced costs but can also provide favourable environmental implications like decline in eutrophication and GHG emissions. Even though regarded as a suitable alternative, the process is still overshadowed due to the unwanted cross-contamination problems, seasonal variation in yield and metabolite content, thus necessitating further research in the arena (Li et al., 2019). Though the use of biochemically rich microalgal biomass as biofertilizers is a common approach as demonstrated by Renuka et al. (2016), the studies related to the growth of microalgae in wastewater as a source of biostimulants are extremely limited. Recent study by Supraja et al. (2020b) showed the efficacy of biostimulants from the wastewater grown microalgal consortium dominated by *Chlorella* sp., along with *Scenedesmus* sp., and *Synechocystis* sp., utilized as seed priming and foliar spray agent. It is essential to optimize the process conditions, remove the social taboos and enigmas linked wastewater utilization for the food production process to increase its applicability in near future.

Anaerobic liquid digestate generated from the biogas plants is a rich source of ammonium (~3000 mg/L) which if uncontrollably spread over land as a direct fertilizer might have toxic environmental effects, also causing tremendous eutrophication (Wang et al., 2019). This liquid digestate, however can easily be pre-treated and utilized for algal cultivation under the waste to wealth scheme (Fuentes-Grünewald et al., 2021). A recent study by Jiang et al. (2018) demonstrated successful recovery of 51.6–57.8% ammonium and 76.6–86.8% phosphate from the anaerobic digestate using *S. obliquus* and *C. vulgaris* respectively. Though, the use of anaerobic digestate for algal cultivation has been recently practised by several researchers (Uggetti et al., 2014; Xia and Murphy, 2016; Stiles et al., 2018), the utilization of the algal biomass as a source of biostimulants are least explored. The North-West Europe, Interreg have recently developed a project ALG-AD under the corporate social program by European Commission to achieve sustainability

aiming to integrate the anaerobic digestate from food and farm waste to produce algal biomass that can be processed into value-added feed or biostimulants (ALG-AD [WWW Document] (2020)). However, there has been no detailed reports until now showing the biostimulatory activity of the algal biomass grown from anaerobic digestate. Although, the integrated treatment of waste anaerobic effluent via algal growth into value-added products is considered an essential technique for resource recovery, more insights into the biochemical characterization and metabolite composition of algal biomass grown in anaerobic digestate must be further researched to promote its use.

Further, the algal biorefinery platform aims to systematically and sequentially utilize the algal biomass to generate multiple products within a single process to improve the economic feasibility. Safi et al. (2014) utilized high pressure homogenization for disrupting *T. suecica* followed by ultrafiltration for obtaining starch and pigments, with the sugars and proteins separated in the permeate. Researchers have also combined freeze thawing and membrane filtration to obtain high value compounds e.g., polysaccharides, proteins and lipids from *Scenedesmus* sp. (Ansari et al., 2017); eicosapentaenoic acids; carbohydrates and fucoxanthin from *P. tricornutum* (Gao et al., 2017). Though the downstream processing strategy has been combined to obtain multiple products, there is no supporting data for the process scale-up and economic feasibility of these approaches. Alternatively, it might be advantageous to combine the biorefinery strategies where these high value products could be extracted and the residual biomass can be converted through hydrothermal conversion processes into bioenergy. As represented in Fig. 3, the waste streams (wastewater / anaerobic digestate) along with the flue gas from industries, can be utilized to grow microalgae (Step 1), which can then be subjected to drying and physico-chemical extraction of bioactive elements (Step 2); the bioactive elements or the biostimulatory compounds obtained can then be used as an alternative to the synthetic chemicals/fertilizers to produce healthy food (Step 3); the food wastes generated can be utilized as substrate for anaerobic digestion (Step 4) to generate electricity that can be used as a source of renewable energy in Steps 1 and 2, apart from culturing microalgae with anaerobic digestate as a nutrient source. Additionally, the residual biomass obtained in Step 2, can be thermo-chemically processed via hydrothermal carbonization into hydrochar that could be potentially utilized as either fuel/adsorbent/soil conditioning agent based on its properties. This algal biorefinery based circular bioeconomy concept would also facilitate the integration of the low volume high value product based markets with that of the high volume low value bioenergy markets, where the economic feasibility might be suitable and more attractive.

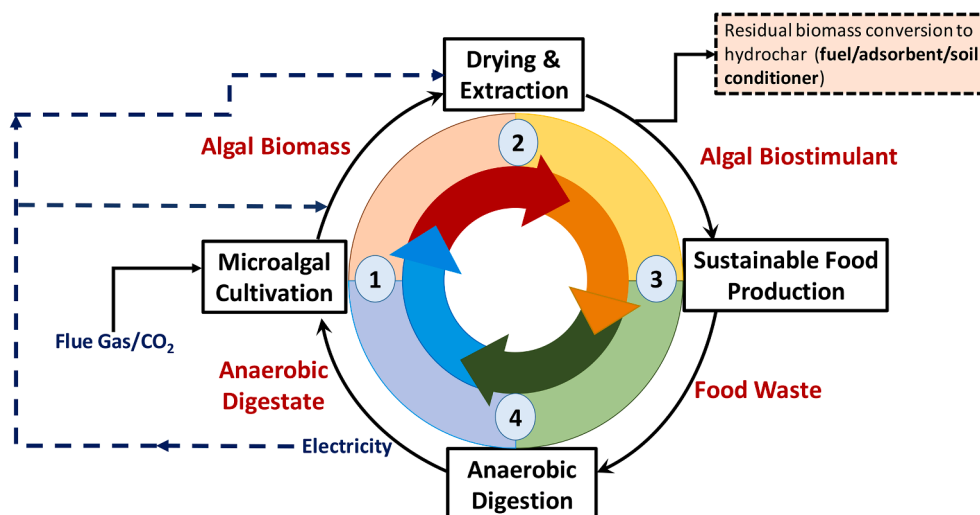


Fig. 3. Circular bioeconomy approach for the assimilation of microalgal biorefinery with the production of biostimulants.

6.2. Rapid screening and metabolic profiling of microalgal strains

Most biostimulant studies done until now have been restricted to single strains or mixed consortium consisting mostly fresh water species like *Chlorella* sp., *Scenedesmus* sp., and marine water species like *Nannochloropsis* sp., *Tetraselmis* sp., and *Porphyidium* sp. (Kapoor et al., 2021). A vast majority of strains still remain unexplored due to the lack of information on the biochemical and phytochemical content to establish their biostimulatory action. Isolation and screening of microalgae targeted for biostimulatory compounds are limited by the lack of reliable and sufficient data on the underlying metabolic pathways governing the production of these bioactive compounds (Chiaiese et al., 2018). Also, the synergistic or antagonistic interaction of microalgal extracts with the microbiome of the rhizosphere, promoting nitrogen fixation is yet to be studied. The lack of underlying metabolic principles hinders the use of genetic, metabolic and transcriptomic approaches for rapid high throughput screening of microalgae with biostimulatory properties (Bulgari et al., 2019). As a breakthrough into the existing research gap, a recent study by Ugena et al. (2018) postulated a multi-trait phenotyping process to screen and categorize the microalgal biostimulants as plant growth promoters or inhibitors or even stress alleviators based on their mode of action. More such biotechnological innovations, integration of the metabolomics approach and forward genomics approach are crucial to understand the metabolic interactions of the bioactive compounds and the host plant to screen their presence or detect these phenotypes in novel microalgal strains.

6.3. Addressing environmental and techno-economic constraints

Uncertainties associated with resource utilization during microalgal cultivation for biostimulants often delimits its commercialization. Even though the water source has no role over the metabolite interaction, the freshwater cultivation possesses high water footprints; and the use of marine microalgae add up to the downstream process costs of washing thus, causing water scarcity (Mutale-Joan et al., 2020). Still, the system for extracting the biostimulatory compounds from marine microalgae needs to be located near saline source, which if located at a very distant site might add up to the transportation costs. Also, the huge need of freshwater sources has led to the opposition by environmentalists debating the need of water use and diversion for the growth of agricultural crops than towards microalgae cultivation. However, this challenge could be averted by hypothesizing that microalgal biostimulants can provide drought resistance to the plants. Partially, if the algal biomass obtained during cultivation could be diverted into two

streams, where half of the harvested biomass can be processed for biostimulant extraction and, the other part of the biomass as a whole (wet algae) could be applied into the soil improving the water holding capacity of soil, thereby declining the need of water during crop production. Similarly, the recycling of the growth medium is expected to be an essential approach in reducing the costs linked with residual water treatment before disposal, apart from reducing the overall water footprints if linked with the cultivation process. Since, a substantial amount of unused nutrients will also be recirculated, this process is also expected to reduce the costs associated with the nutrient demands (Thomassen et al., 2016). Colocation of microalgal cultivation system within industrial premises is also considered an essential approach to reduce the costs associated with land as well as sequester CO₂ thereby, decreasing the GHG emissions, with additional revenues obtained from the sale of wet algal biomass as plant growth stimulant (Behera et al., 2019b). The incorporation of the biorefinery concept can also bring down the investment costs making the overall process cost efficient and attractive during commercialization (Thomassen et al., 2016). Overall, there is an utmost need for expanding the techno-economic and life-cycle assessment tools to provide better insights to improve the economic profitability with lower ecological impacts.

7. Prospects and future directions

Although microalgal biostimulants are deemed as the cost-effective eco-friendly substitute of the synthetic chemical based biostimulants, the growth and commercialization of markets for these products are significant lagged behind compared to the macroalgal counterparts. The strength-weakness-opportunity-threat (SWOT) analysis as illustrated in Fig. 4 presents the prospects and the pros of these techniques emphasizing the need for safe and healthy food without residual chemical contaminants. Algal biostimulants with a diverse metabolite composition, though have a huge scope of market expansion, presently these products occupy a very small market volume compared to other competitors. Further, increasing the scale or volume of production is also expected to saturate the market. Thus, there is an ardent necessity to consider intervention from different aspects at various mode of production until the market entry stage (Fig. 5). Some of the arguments to be, considered for guiding future research needs are presented below:

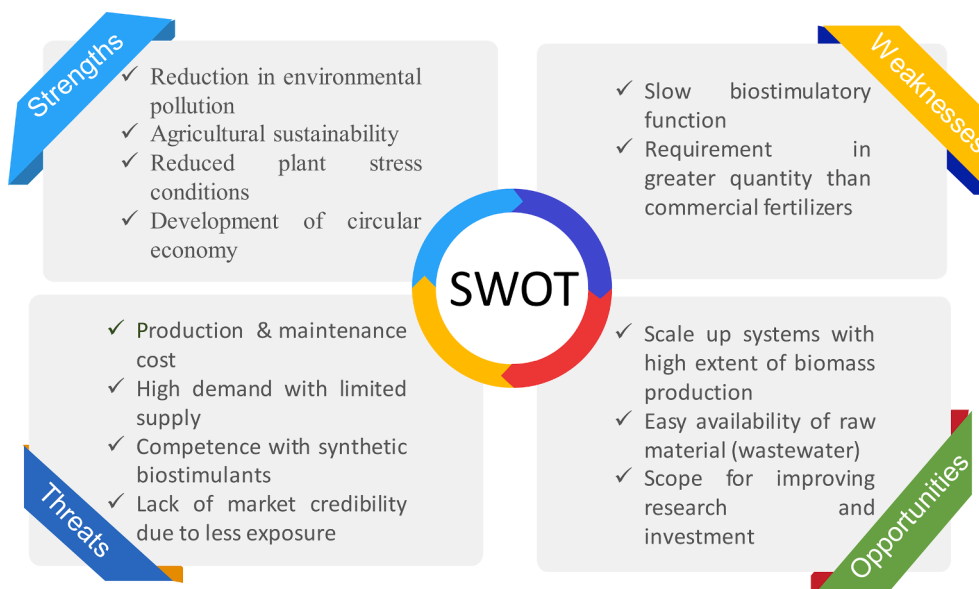


Fig. 4. Strength-Weakness-Opportunity-Threat (SWOT) analysis of microalgal biostimulants markets.

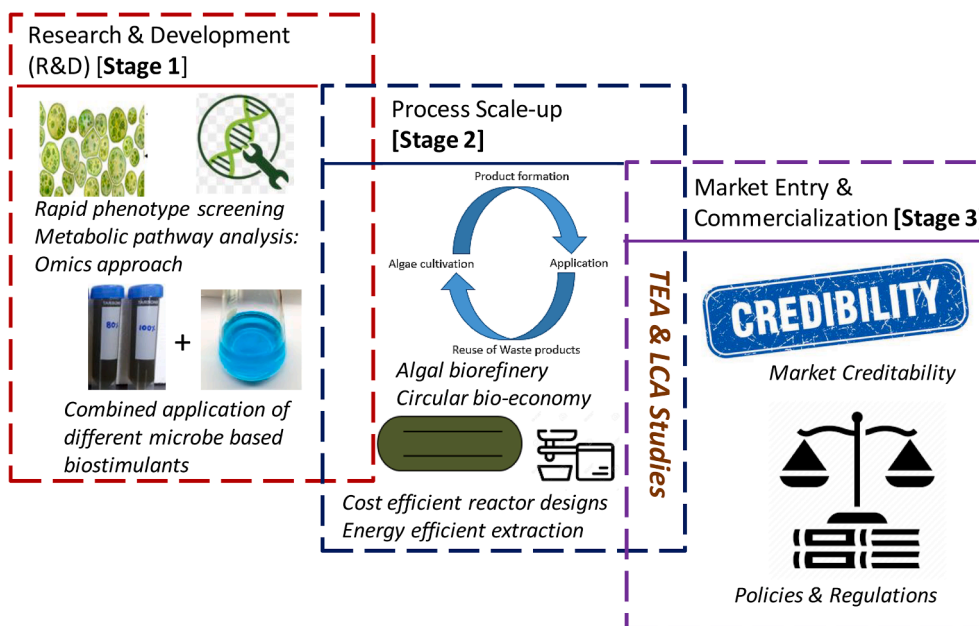


Fig. 5. Systematic step by step approach for the expansion and commercialization of biostimulants from microalgae.

8. Research and development (R&D)

- Development of high throughput phenotype screening technologies to identify robust microalgal strains capable of sustaining in waste resources with high bioactive metabolite content.
- Integration of molecular, metabolomics and synthetic biology aspects linked with the biochemical/metabolite accumulation during growth to identify genetic approaches for strain engineering.
- Understanding the interaction of the metabolite at cellular level in different parts of plant or with the microbiome associated in the rhizosphere to identify new metabolites for targeted application.
- Blending of microalgal biostimulants with similar bioactive compounds from other microbial sources in appropriate ratio to study the synergistic/antagonistic interactions during the plant growth and microbiome composition.

9. Scale up of algal cultivation / field scale application of biostimulants

- Incorporation of biorefinery approach starting from cultivation in waste nutrient streams to extraction of multitude of products during the downstream processing.
- Devise cultivation strategies and reactor designs that could be utilized at ease with less cross-contamination and need of additional product sterilization.
- Utilization of mild, eco-friendly and energy efficient drying and extraction techniques with less detrimental impact over the metabolite bio-efficacy.
- Assessment of the location specific seasonal impacts on algal productivity and metabolite accumulation.
- Rigorous utilization of tools for economic and life-cycle impacts of biorefinery scenarios specifically for biostimulant production.

- Field scale trials to cross-validate the application type (seed treatment; foliar spray; root application) dosage and frequency based on the seasons, crop type and geographical location.
- Evaluate the nutritional quality, quantity and safety aspects of algal biostimulant actions on fruits and vegetables.
- Assess the impact of long term usage of biostimulants over the environmental standards and ecological biodiversity.
- Cost efficient upstream and downstream bioprocess strategies to reduce the time to market windows for facilitating easier product entry.

10. Market entry, policies and regulations

- Preventing market saturation and comply with the competitors and consumer demands via controlling the production magnitude through flexible biorefinery concepts.
- Targeting a particular niche of consumers or farmers to facilitate market entry, colonization and earn profitability.
- To establish a standardized, certain, safe and secured environment promoting in the regulatory frameworks for patentability.
- Framing policies to ensure easier compliance and certifications with respect to the product safety and organic certification, especially while utilizing waste streams/resources as nutrients promoting safe use of microalgal stimulants.

Apart from the need to promote further research to address the technical externalities constraining the commercialization of microalgal biostimulants, there is a prudent requirement for consumer awareness to promote its use and real time implementation. Transition from the mindset of considering the microalgal biostimulants from the present scenario of just a “eco-friendly substitute for chemical fertilizers” to a “product of unique attribute that is essential plant growth promoting agent” must be prophesized. Ecological benefits and monetary advantages must be portrayed to drive the interest of funding agencies and policy makers. Nevertheless, the researchers and the non-governmental or service agencies hold a tremendous responsibility to aware the end users or farmers about the multitude of benefits to make the real time implementation and commercialization possible in near future.

11. Conclusions

Microalgal biostimulants hold an enormous ability to achieve resilience and sustainability in the environment. With additional advantage of striving on waste resources, microalgae can be efficiently tailored to accumulate bioactive metabolites in desired concentration that could have biostimulatory action over the plant metabolism. In spite of the advantages, the microalgal biostimulant markets still remains dormant and is lagging behind its competitors. The use of omics approaches, rapid phenotyping techniques can be utilized to screen robust algal strains and metabolites integrated in a biorefinery platform to address the enviro-techno-economical externalities, remove the regulatory barriers to gain consumer trust making the product acceptable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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