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## Cyanobacterial/algal biofertilizers as plant growth stimulants for green sustainable agriculture

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### Abstract

Population of the Earth has been projected to reach ~9.7 billion by 2050 and Asia and Africa would be the major contributors to this enhanced population. This increased population is directly related with higher demand of food security in very near future. Modern agricultural management is heavily dependent on the fertilizers for increased crop production, but increasing use of chemical-based and inorganic fertilizers may be a serious threat to the environment and human health. Microalgae have biostimulant and biofertilizer properties which are attracting the interest of farmers and agrochemical industries. Application of microbes such as cyanobacteria, microalgae, endo/ecto-mycorrhizal fungi, rhizobacteria, and others serves as an ecofriendly approach for sustainable agriculture practices. Biostimulants are the products derived from organic matter that are applied in small quantities and are able to stimulate the development and growth of several crops under stressed as well as optimal environmental conditions. Plant biostimulants encompass diverse organic and inorganic substances (humic acids and protein hydrolysates) as well as prokaryotes (plant growth promoting bacteria) and eukaryotes such as mycorrhiza and macroalgae (seaweed). Biofertilizers are products containing living microorganisms or natural substances that are able to improve chemical and biological soil properties, stimulating plant growth, and restoring soil fertility. Microalgae and cyanobacteria (blue-green algae), are attracting interest from scientists, extension specialists, private industry and plant growers because of their versatile nature, simple unicellular structure, high photosynthetic efficiency, ability for heterotrophic growth, adaptability to domestic and industrial wastewater, amenability to metabolic engineering, and possibility to yield valuable co-products. This review article focuses on the research achievements on cyanobacteria and microalgae based plant biofertilizers and biostimulants in the agricultural applications. The challenges to commercializing these kinds of biofertilizers are also discussed.

**Keywords:** Biofertilizer, biostimulants, cyanobacteria, microalgae, soil fertility, sustainable agriculture

### 1. Introduction

To overcome the challenge of increasing population, the World Health Organization has suggested a doubling of food production by 2050, while the United Nations has suggested a 50% increase in the global food production by 2030. The productivity gains resulted from the “Green Revolution” have essentially reached a plateau, and feeding the increased global population is further challenged by limited availability of agriculture land. Nevertheless, a production system which has a higher productivity but requires a small land area and time for cultivation would be the requirement of the future agriculture [1-2]. In recent years, microalgae and cyanobacteria, have emerged as potential candidates for their application in development of environment friendly and sustainable agricultural practices [3-4]. These oxygen evolving photosynthetic organisms do not compete for arable land for their cultivation. Cyanobacteria, which can be cultivated using seawater, require residual nutrients for high areal productivity and have high protein and reasonable amount of carbohydrate as well as lipid contents per gram of their biomass [5-7]. Globally about 25 Gt a<sup>-1</sup> of carbon can be fixed into energy-dense biomass by cyanobacteria using atmospheric CO<sub>2</sub> and solar energy [8]. Therefore, generation of microbiological energy through massive solar energy transformation permits harvesting of various forms of eco-friendly energy reserves [9].

The aforementioned characteristics make microalgae and cyanobacteria potential microorganisms for their application as feedstocks for sustainable production of food and non-food commodities, including valuable chemicals and bioenergy [2, 10-12].

## 2. Cyanobacteria as Biofertilizers

It is very expensive to produce inorganic nitrogen fertilizers due to the requirement of large amount of fossil-fuel energy. This necessitated the development of alternate, sustainable and cost-effective biologically available nitrogen resources which can fulfill the nitrogen demand of agriculture in sustainable manner [13]. For this purpose biological systems have been identified which can fix atmospheric dinitrogen [14]. Biological nitrogen fixation contributes  $\sim 2 \times 10^2$  Mt of nitrogen annually [15]. According to Metting [16], the total nitrogen fixation can be  $\sim 90$  kg N ha<sup>-1</sup> y<sup>-1</sup>. Symbiotic and free-living eubacteria, including cyanobacteria, are two groups of nitrogen fixing organisms. The free-living cyanobacteria fix  $< 10$  kg of N ha<sup>-1</sup> y<sup>-1</sup>, however, annually  $\sim 10$ -30 kg of N ha<sup>-1</sup> is fixed by dense mats of cyanobacteria [17-18]. Therefore, cyanobacteria constitute an important component of naturally available biofertilizers [14, 19]. Rice production in tropical countries mainly depends on biological N<sub>2</sub> fixation by cyanobacteria which are a natural component of paddy fields [14]. In these cultivated agriculture systems, annually  $\sim 32$  Tg of nitrogen is fixed by biological nitrogen fixers [3], and cyanobacteria add about 20-30 kg fixed nitrogen ha<sup>-1</sup> along with organic matter to the paddy fields [20-21]. Cyanobacteria also make symbiotic associations with different photosynthetic and non-photosynthetic organisms such as algae, fungi, diatoms, bryophytes, hornworts,

liverworts, mosses, pteridophytes, gymnosperms, and angiosperms [22-23]. Several heterocystous cyanobacterial genera such as *Anabaena*, *Nostoc*, *Nodularia*, *Scytonema*, *Schytonema topsis*, *Chlorogloea*, *Cylindrospermum*, *Mastigocladus*, *Calothrix*, *Anabaenopsis*, *Aulosira*, *Tolypothrix*, *Haplosiphon*, *Camptylonema*, *Stigonema*, *Fischerella*, *Gloeotrichia*, *Chlorogloeopsis*, *Rivularia*, *Nostochopsis*, *Westiellopsis*, *Wollea* and *Westiella* have been shown to be efficient N<sub>2</sub> fixers [24].

Table 1 contains a list of potential cyanobacteria which can be used as biofertilizers in agricultural fields [14]. For the first time, Fritsch, [25] studied the abundance and importance of cyanobacteria with respect to maintenance of soil fertility of paddy fields through biological nitrogen fixation, which was afterwards recognized by several other workers [26-28]. Generally, for algalization of the rice fields, mixed cyanobacterial cultures of free-living forms are used [29-30]. The water fern *Azolla* harbors *Anabaena azollae* in its fronds and the cyanobacterium releases ammonium into the water when paddy fields are inoculated with foam-immobilized *A. azollae* strains [31]. Significant increase in grain yield, biomass and nutritive value of rice can be achieved by inoculating *Anabaena doliolum* and *A. fertilissima* in paddy fields with or without urea [32]. In addition to rice crop, cyanobacterial biofertilizers can also enhance the yield, shoot/root length, and dry weight of wheat crops [33-35]. Inoculation of soil with various cyanobacterial strains like *Nostoc carneum*, *N. piscinale*, *Anabaena doliolum* and *A. torulosa* results in significantly higher acetylene reducing activity [19]. Additionally, the acetylene reducing activity is highest at harvest stage when wheat fields are inoculated with an *Anabaena-Serratia* biofilm along with rock phosphate [36].

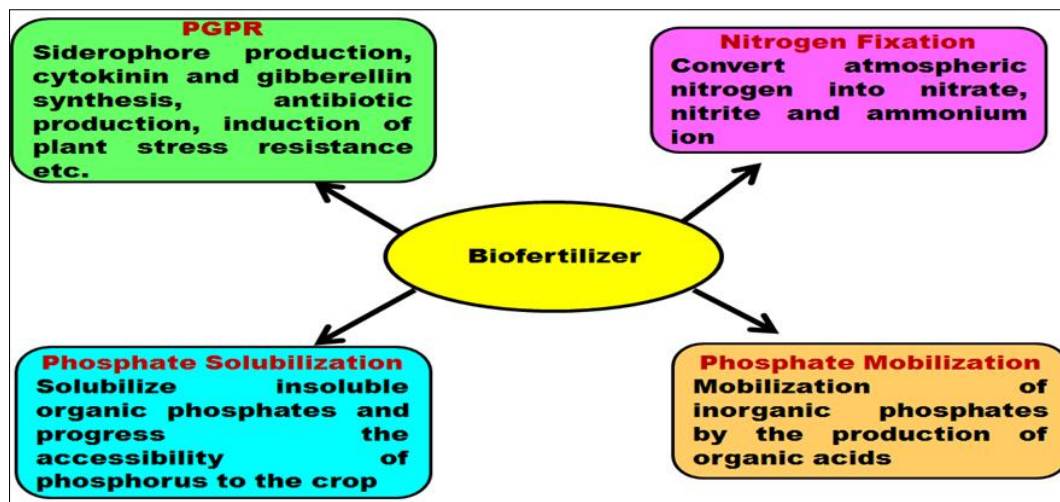
**Table 1:** List of nitrogen-fixing cyanobacteria important for their application in biofertilizer industry (Adapted from Vaishampayan *et al.* [14])

<b>Filamentous</b>
<b>Heterocystous</b>
<i>Anabaena</i> , <i>Anabaenopsis</i> , <i>Aulosira</i> , <i>Calothrix</i> , <i>Camptylonema</i> , <i>Chlorogloea</i> , <i>Chlorogloeopsis</i> , <i>Cylindrospermum</i> , <i>Fischerella</i> , <i>Gloeotrichia</i> , <i>Haplosiphon</i> , <i>Mastigocladus</i> , <i>Nodularia</i> , <i>Nostoc</i> , <i>Nostochopsis</i> , <i>Rivularia</i> , <i>Scytonema</i> , <i>Scytonematopsis</i> , <i>Stigonema</i> , <i>Tolypothrix</i> , <i>Westiella</i> , <i>Westiellopsis</i> , <i>Wollea</i>
<b>Non-heterocystous</b>
<i>Lyngbya</i> , <i>Microcoleus chthonoplastes</i> , <i>Myxosarcina</i> , <i>Oscillatoria</i> , <i>Plectonema Boryanum</i> , <i>Pseudoanabaena</i> , <i>Schizothrix</i> , <i>Trichodesmium</i>
<b>Unicellular</b>
<i>Aphanothece</i> , <i>Chroococciopsis</i> , <i>Dermocarpa</i> , <i>Gloeocapsa</i> , <i>Myxosarcina</i> , <i>Pleurocapsa</i> , <i>Synechococcus</i> , <i>Xenococcus</i>

The cyanobacteria based biofertilizers are cost-effective as they cost one third to that of chemical fertilizers [19]. In addition to nitrogen fixation, cyanobacteria also contribute to mobilization of inorganic phosphates through excretion of organic acids and extracellular phosphatases [37-38]. Cyanobacteria solubilize and mobilize the insoluble organic phosphates and improve the availability of phosphorus to the crop [39-40]. The humus content generated after death and decay of cyanobacteria creates strong reducing condition in soil which improve the soil structure and fertility [41]. Different cyanobacterial strains are known to produce plant growth hormones and siderophores, and therefore, cyanobacteria can affect the development and productivity of

crops [42-43].

The exo-polysaccharides secreted by cyanobacteria induce aggregation of soil particles which improve the soil structure and fertility by enhancing the accumulation of organic content and water accumulation [2]. These findings collectively support the importance of cyanobacteria as biofertilizers, and methods have been developed for their cultivation and utilization in fertilizer industry [44-46]. In general, biofertilizers are defined as the organic compounds from living microorganisms to promote the growth of seeds, plants, or soil bacterial consortia by essential nutrients such as nitrogen, phosphate, potassium and other ineral nutrients (Figure 1) [47-49].



**Fig 1:** Role of biofertilizers in maintaining soil health

They should be distinguished from the NPK fertilizers, which are based on Nitrogen (N), Phosphorous (P), and Potassium (K), the three main nutrients necessary in high quantities for healthy growth of higher plants. Biofertilizers are classified by the microorganisms and the benefits achieved by their application: nitrogen-fixators; phosphates and potassium solubilizing biofertilizers; phosphorus-mobilizing biofertilizers and biofertilizers for secondary macronutrients, zinc and iron solubilizers, plant-growth-promoting rhizobacteria (PGPR), and compost [47, 50]. The remarkable features of biofertilizers include improved crop productivity per area in a relatively short time; reduced amounts of energy consumption and contamination of soil and water; increased soil fertility; and promoted antagonism and biological control of phyto-pathogenic organisms [51]. Biofertilizers will provide renewable and environmental friendly solutions for modern agriculture, especially in the form of integrated nutrient management (INM) and integrated plant nutrition system (IPNS), which may lead to sustainable economic development [52-53]. However, several challenges are associated with biofertilizers, such as the short period of shelf life (3-4 months) and the conditions required for storage (cool temperatures because of their temperature sensitivity) [54]. Recent studies have indicated that different types of photosynthetic microorganisms such as cyanobacteria and microalgae can be used as biofertilizers and soil conditioners [55-56]. Microalgae are generally divided into Chlorophyta (green algae), Rhodophyta (red algae), Phaeophyta (brown algae), Euglenophyta, Pyrrophyta, and Chrysophyta. Cyanobacteria are the only photosynthetic prokaryotes able to produce oxygen [57]. There are around 30,000 species of both unicellular microalgae and more complex multicellular organisms [58]. Among them, 150 genera and more than 2000 species of microalgae have been listed [59].

Research on industrial applications of algae has been conducted since early 1950s, when productivity and yields were first studied in mass culture [60]. The main industrial benefits of algae are their ability to grow with minimum freshwater inputs and utilize lands that are otherwise agriculturally non-productive. Algae are widely considered to have major influence on essential ecosystem services since they can be cultivated in wastewater and agricultural runoff, recovering excess nutrients and reclaiming water for further use. They can also reduce greenhouse gas emissions by sequestering carbon dioxide and nitrous oxides from

industrial sources [61]. Cyanobacteria are considered the simplest, living autotrophic microorganisms. These organisms are capable of building up food materials from inorganic matter and are widely distributed in the aquatic environment [62]. Several unique features of cyanobacteria, such as water-holding capacity, short generation time, ability to fix atmospheric  $N_2$ , and adaptation to extreme conditions, make them an effective biofertilizer source to improve soil physico-chemical properties [3, 62]. Cyanobacteria can also secrete plant growth hormones as secondary metabolites, promote the transport of nutrients from soil to plants, cause agglomeration of soil, and improve the chemical properties of the soil [63]. Their diverse morphology and physiological properties enable wide distribution in the ecosystem and tolerance to environmental stresses [64]. This review explores photoautotrophic microorganisms for functions that facilitate the development of biological fertilizers in the agricultural sector. This paper is aimed at reporting developments in the processing of microalgal biostimulants (MBS) and biofertilisers (MBF), summarizing the biologically-active compounds, and examining the researches supporting the use of MBS and MBF for managing productivity and abiotic stresses in crop productions. Microalgae are used in agriculture in different applications, such as amendment, foliar application, and seed priming. MBS and MBF might be applied as an alternative technique, or used in conjunction with synthetic fertilizers, crop protection products and plant growth regulators, generating multiple benefits, such as enhanced rooting, higher crop yields and quality and tolerance to drought and salt. Worldwide, MBS and MBF remain largely unexploited, such that this study highlights some of the current researches and future development priorities. Plant biostimulants (PBs) attract interest in modern agriculture as a tool to enhance crop performance, resilience to environmental stress, and nutrient use efficiency. On the other hand, large-scale biomass production and harvesting still represent a bottleneck for some applications. Although it is long known that microalgae produce several complex macromolecules that are active on higher plants, their targeted applications in crop science is still in its infancy. This paper presents an overview of the main extraction methods from microalgae, their bioactive compounds, and application methods in agriculture. Mechanisms of biostimulation that influence plant performance, physiology, resilience to abiotic stress as well as the plant microbiome are also outlined. Considering current

state-of-the-art, perspectives for future research on microalgae-based biostimulants are discussed, ranging from the development of crop-tailored, highly effective products to their application for increasing sustainability in agriculture (Figure 2).

### 3. Algae-Based Biofertilizers

Algae biotechnology research in the field of biofertilizers has increased in recent years. The majority of the studies published recently used *Chlorella* sp. as the model system (Table 2) [65-74]. The most striking potential of algae is that they can survive even in the presence of highly concentrated organic and inorganic chemicals in varying waste streams which are toxic to living organisms. This is important in enabling more sustainable and efficient production in

agriculture. Microalgae can be autotrophic or heterotrophic. As solar conversion in some microalgae species is very efficient, the most common procedure for cultivation of this microorganism is presently the autotrophic growth [75]. The basic cultivation system consists of open-ponds used for food supplement and antioxidant production, with highly variable productivity depending on species and environmental conditions [76]. Open system cultivation of microalgae is thus limited to certain robust species, such as *Spirulina* spp., *Dunaliella* spp., and *Chlorella* spp., that are able to grow under extreme conditions. Reduction of growing area and protection against potential contamination can be obtained in closed-ponds, referred to as photobioreactors. This type of cultivation method is often used for the production of high added-value molecules, such as pharmaceutical compounds.

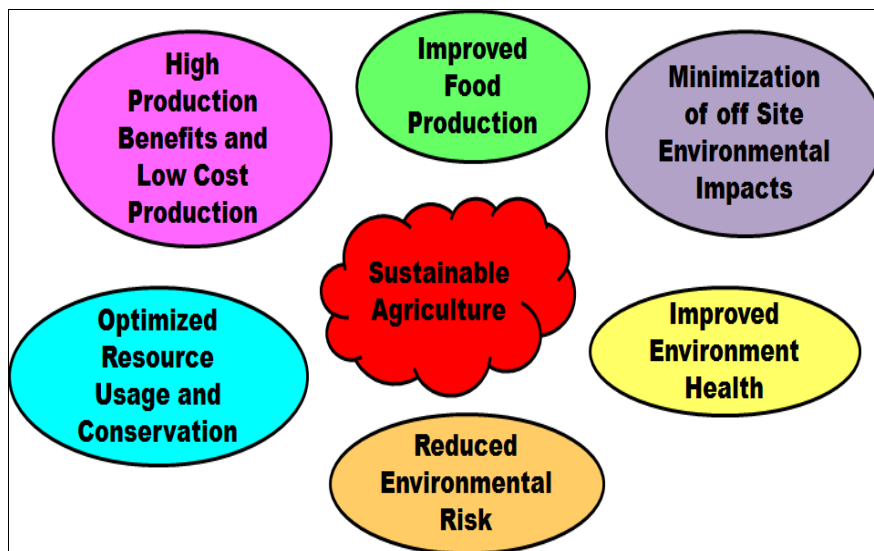


Fig 2: Sustainable agriculture and its various aspects

Table 2: Microbial biofertilizers and their applications

Species	Formulation	Application	Reference
<i>Spirulina platensis</i>	Cyanobacterial biofertilizer -intact cells (Spirufert_bio-fertilizer)	Foliar treatment on eggplant	Dias <i>et al.</i> [77]
<i>Spirulina platensis</i>	Cyanobacterial biofertilizer -intact cells after aquaculture wastewater remediation for nitrogen fixation	Spread biomass for leafy vegetables	Wuang <i>et al.</i> [78]
Consortium ZOB1	consortium biofertilizer -intact cells as biostimulator for crops	Not mentioned	Zayadan <i>et al.</i> [79]
<i>Anabaena</i> sp. <i>Aulosira</i> sp. <i>Cylindrospermum</i> sp. <i>Nostoc</i> sp. <i>Tolypothrix</i> sp.	Each one exploitable as cyanobacterial biofertilizer -intact cells for nitrogen fixation and indole acetic acid (IAA) growth promoting substance	Wet land rice cultivation	Chittora <i>et al.</i> [80]
Iran native nitrogen-carbon fixing cyanobacteria and bacteria	cyanobacterial biofertilizer/other biofertilizer -intact cells for nitrogen and carbon fixation	Spread to erosion prone soil	Kheirfam <i>et al.</i> [63]
Nitrogen-fixing cyanobacteria	cyanobacterial biofertilizer -intact cells for nitrogen fixation	Spread on soil for rice cultivation amended with fly ash	Padhy <i>et al.</i> [81]
<i>Frankia</i> Hsli10	cyanobacterial biofertilizer -possible intact cells application for saline soil	Not mentioned	Srivastava and Mishra [53]
<i>Chlorella</i> sp.	Micro algae (MA) biofertilizer - suspensions of microalgae culture and sterile filtrates from wastewater treatment	Spread to agricultural soil	Evan <i>et al.</i> [67]
<i>Chlorella vulgaris</i>	MA biofertilizer - cells digestate of anaerobic reactors after growth in wastewater	Not mentioned	Doğa-Subaşı and Demirel [66]
<i>Chlorella vulgaris</i>	MA biofertilizer	dry or liquid microalgae biomass	Ozdemir <i>et al.</i> [72]

		Dry and liquid algae widespread to agricultural soil; foliar spray	
<i>Chlorella pyrenoidosa</i>	MA biofertilizer - cellular biomass after im mobilizing for dairy effluent treatment	Spread on rice seeds	Yadavalli and Hegggers <sup>[74]</sup>
<i>Acutodesmus dimorphus</i>	MA biofertilizer - cellular extracts, in distilled water and dry Biomass	Spread to agricultural soil; foliar spray	Garcia-Gonzalez and Sommerfeld <sup>[68]</sup>
<i>Microcystis aeruginosa</i> MKR 0105 <i>Anabaena</i> PCC 7120 <i>Chlorella</i> sp.	Blue-green algae (BGA) plus MA biofertilizer - intact cells with limited use of YaraMila Complex synthetic fertilizer	Triple foliar	Grzesik <i>et al.</i> <sup>[70]</sup>
<i>Chlorella vulgaris</i> <i>Scenedesmus obliquus</i> "Consortium C"	Consortium biofertilizer - possible use of cellular bio mass growth in wastewater	Not mentioned	Gouveia <i>et al.</i> <sup>[69]</sup>
<i>Phaeodactylum tricornutum</i> <i>Pavlova lutheri</i>	MA biofertilizers - possible use of cellular biomass growth in mineral medium and agro-industrial ultra-filtrate	Not mentioned	Veronesia <i>et al.</i> <sup>[73]</sup>
Consortium of 21 microorganisms	Consortium biofertilizer - possible use based on study of morphological and phylogenetic diversity	Not mentioned	Hernandez Melchor <i>et al.</i> <sup>[71]</sup>
Native microalgae "Consortia 01" and "Consortia 12"	MA biofertilizer - possible readily use of biomass after polishing treatment of municipal waste water	Not mentioned	Beltrán-Rocha <i>et al.</i> <sup>[65]</sup>

However, the main disadvantage of photobioreactors is the high capital cost for designing and operating. A viable alternative for growing microalgae is in heterotrophic conditions exploiting existing industrial bacterial-bioreactors <sup>[82-85]</sup>. The main advantages of this cultivation system is the high cell concentration, which can reach up to 100 gL<sup>-1</sup>; in photobioreactors, the maximum density is around 40 gL<sup>-1</sup>, even lower in open-ponds (c. 10 gL<sup>-1</sup>). A recent sustainable energy based strategy for growing microalgae relies on using wastewater of industrial, domestic and agricultural origin in bioreactors that allow for the removal of contaminants during the production of microalgal biomass. Therefore, microalgae have the potential to reduce the negative discharges to the environment by, for instance, re-using nutrients and products and valorizing waste from different sources, including those related to agriculture.

### 3.1 Soil Fertility

Algal biomass formation from wastewater treatment can add value to land use as a biofertilizer, although not much information is available on how it may affect soil nutrient dynamics. Research focused on the indigenous species of *Anabaena* has showed the ability of this strain to promote soil fertility while decreasing soil density <sup>[86]</sup> even in land with herbicide residuals and limited water supply <sup>[87]</sup>. Similarly, Marks *et al.* <sup>[88]</sup> investigated the effects of unicellular green algae on the soil organic carbon using microalga *Chlorella* sp. grown in the liquid slurry. Their results indicated that photoautotrophic growth of *Chlorella* sp. was 3.5 times higher than that grown in dark and culture filtrates without algal cells, and soil respiration was significantly increased <sup>[67]</sup>. Another interesting research area is the study of algal and bacterial consortia in the biofertilizer application. In fact, it could not only be more efficient in detoxification of pollutants and removal of nutrients from wastewaters compared to the use of individual microorganisms, but such consortia could also allow maximum use of available N, P, and K in the soil. The pollutant abatement between algae and bacteria would lead to the success of consortium engineering <sup>[14, 36, 71, 79, 89]</sup>. Furthermore, literature suggests that algae/bacteria consortia have great potential for soil amendment of marginal lands,

helping to transform them into agricultural soil.

### 3.2 Nitrogen Fixation

One reason to use cyanobacteria as biofertilizers is based on their nitrogen-fixing ability. Cyanobacteria convert inorganic nitrogen (N<sub>2</sub>) from the air into organic nitrogen that can be easily utilized by higher plants <sup>[59]</sup>. Efforts to use cyanobacteria to promote rice growth have been made both in India and Chile. Local cyanobacterial strains in Chile have shown to increase nitrogen accumulation efficiency in rice paddles. Vaishampayan *et al.* <sup>[14]</sup> recommended that the *Azolla-Anabaena* (the free-living cyanobacteria *Anabaena* and the water fern *Azolla*) symbiotic N<sub>2</sub>-fixing complex be considered self-renewable natural nitrogen resources to reduce inorganic N requirements to the bare minimum. The cyanobacterium *Tolypothrix* sp. was found to produce bioproducts in tropical regions by using low nitrogen containing water sources <sup>[90]</sup>.

According to a comparative study with N<sup>15</sup>-labelled fertilizer and indigenous cyanobacteria, N<sub>2</sub> recovery by the soil-plant system from cyanobacteria was higher than that from chemical fertilizer <sup>[91]</sup>. This algal strain was highly capable of increasing the growth of rice plants due to its nitrogen-fixation ability <sup>[74]</sup>. In another work, following treatment with immobilized *Chlorella pyrenoidosa*, dairy waste water effluent used as a biofertilizer increased rice plants' root and shoot length by 30% <sup>[74]</sup>. In another work, the inoculants of *Anabaena laxa* and *Anabaena-Rhizobium* consortium were used to formulate biofilm in chickpea cultivation. The *A. laxa* inoculation for the biofilm led to 50% higher grain yield (1,724 kg/ha) compared to the control (847 kg/ha) <sup>[74]</sup>. In addition, microbial association (21 different microorganisms containing proteobacteria, bacterioidetes, chlorophyta, etc.) was shown to have a high capacity for N<sub>2</sub>-fixation (10,294 nmol ethylene/g dry weight/h), when used as a biofertilizer <sup>[71]</sup>. A more comprehensive description of cyanobacteria use in agriculture as nutrient supplements can be found in several literatures <sup>[53, 63,77-79, 81,92]</sup> in particular for nitrogen fixation in the wet land rice cultivation.

### 3.3 Production of Plant Growth Biostimulants

Some algal metabolites have been found to stimulate plant growth directly or indirectly by interacting with soil microbes for biomineralization or plant-microbe symbiosis, thereby increasing nutrient availability [93]. To verify this concept, *Lupinus termis* was grown with plant growth-stimulating substances from cyanobacteria and bacteria. The addition of the cyanobacterial filtrate combined with bacterial suspension significantly increased the average germination compared to seeds untreated or treated by hormones (IAA, GA3, and cytokinins). In particular, the germination rates with such treatments were 53.13, 211.48, 129.04, and 104.18% higher in comparison to

1. Untreated seeds
2. Seeds treated with IAA
3. Seeds treated by GA3 and
4. Seeds treated by cytokinins, respectively [94].

Furthermore, algal species isolated from different rice cultivations in the Iranian region were examined for the production of phytohormones that affect plant growth [95]. Under optimal conditions, the cyanobacterium *Nostoc* could produce 8.66 µg/mL IAA, and sprouting was effectively promoted when the infiltrate was added to taro corn field [92]. In another work by Rodríguez *et al.* it was found that the extracellular products of *Scytonema hofmanni* have produced gibberellin-like plant growth regulators, which enabled the hormone homeostasis of rice seedlings under salt stress [43]. On the other hand, Saadatinia and Riahi isolated four species of *Anabaena* strains and tested them in the germination process of rice seeds. The results showed a significant higher germination rate compared to the control [86]. Similar outcomes were shown in the study of Zaydan *et al.* [79] where the cyanobacteria and *Azotobacter* sp. consortium was established.

### 3.4 Biopesticidal Substances

Algae can be used as biocontrol agents with nematicidal effect, [96-98] where extracts and exudates of cyanobacteria have been reported to inhibit hatching and to cause immobility and mortality of juvenile plant parasitic nematodes *in vitro*. Antifungal and antibacterial activities were also studied where culture filtrate has hydrolytic activity against phytopathogens [99-101]. The most economically important fungal pathogen is *Fusarium* sp., and other fungal pathogens have also controlled under above studied. Studies on the biocidal effects of algae have revealed new possibilities to develop novel pest control methods. Future investigations are necessary to validate their spectrum and applications for commercial use.

### 3.5 Tolerance to Extreme Environmental Conditions

Algae are able tolerate various types of environmental stresses. In regard to pesticide resistance, Ningthoujam *et al.*, have found that *Anabaena variabilis* was able to tolerate 100 µg/mL malathion [102]. As an example of salinity tolerance ability, Jha *et al.* demonstrated that cyanobacteria could be negatively affected by Mn and sodium (Na, -30.19%). However, this negative relationship with Na enabled cyanobacteria to be used as an ameliorating agent for salt-affected soil [103]. In another study, the cyanobacterium *Anabaena oryzae* was found to release PO<sub>4</sub><sup>3-</sup> enzymatically under salt stress conditions, suggesting that it could be used in high salinity and alkaline (calcium (Ca<sup>2+</sup>)-rich) soils [104].

Biosynthesis of UV-absorbing molecules, such as mycosporine-like amino acids (MAAs) and scytonemin, as a defence against continued solar radiation in cyanobacteria has been well documented [105-108] and these compounds aids in survival of cyanobacteria under harsh conditions in soils. Sinha and Häder has discussed about the photoprotective defence mechanisms of cyanobacteria against lethal UV-B, which may play a potential role in utilization of cyanobacteria as biofertilizers for the growth of agricultural crops [108]. The main practical problem of commercialized biofertilizer is related to adaptability of active microorganisms in the environment.

### 4. Mutation of Algae for Better Biofertilizers

Some studies have suggested possible solutions for the limitations of biotic and abiotic factors on algae and their performances. Therefore, it is appropriate to use novel approaches to produce cyanobacterial mutants in order to explore the potential of cyanobacteria [109-110]. Singh and Datta [111] demonstrated that *A. variabilis* mutant strains exposed to herbicides were able to resist the herbicide and increase rice growth under outdoor conditions in flooded soils. In another work, the plasmid pRL489 was constructed by Ravindran *et al.* and introduced into *Oscillatoria* MKU 277 by electroporation to establish the gene transfer system in the cyanobacteria [112]. This work has improved the mutational techniques for the development of more powerful and viable biofertilizer strains. A chlorate-resistant mutant (Clo-R) of *Nostoc* ANTH for lack of nitrate was studied by Bhattacharya *et al.* It was observed that heterocyst formation and N<sub>2</sub>-fixation in the presence of nitrate was able to separate nitrate and nitrite transport systems of the mutant. This mutant is supplementary to chemical nitrate fertilizer as a biofertilizer without N<sub>2</sub>-fixation being adversely affected in rice field [113]. Similarly, a nitrogenase derepressed mutant of *Anabaena variabilis* has shown potential for developing biofertilizer for rice production, especially when the rice-production systems aim to minimize environmental pollution from inorganic N fertilizers [114]. In more recent study, cyanobacteria mutant induced by the UV-B has showed tolerance to Cu toxicity, provided that the nitrogen fixation ability was suppressed [115]. Singh *et al.* also improved the *A. variabilis* mutant grown in herbicide(s)-stressed agro-ecosystem [115]. Recent advances in synthetic biology can provide a better solution for handling these challenges and have created a new research area in algal biotechnology.

### 5. Large-Scale Algal Growth

Algal biomass for agricultural applications, in particularly obtained from waste streams, has become an economically attractive investment. Numerous research groups have integrated production of algal biomass with industrial wastewaters bioremediations. For example, Nisha *et al.* and Galhano *et al.* have studied the use of cyanobacteria for both soil fertility and crop protection against residual herbicides. Their potential for improving soil structural stability, nutrient availability and crop productivity has been further exploited under limited water regime which is fundamental for sustainable agricultural management [87, 116]. According to the outcomes of the above studies, indigenous algal strains are more suitable for fertilizer applications. As compared to photobioreactors, open raceways (especially with waste water) are more effective for small capital investments and low power consumption in a large-scale production algal

biomass<sup>[117]</sup>. The important role that algae may play on the elimination of contaminants in various environments is still underestimated<sup>[118]</sup>. Many studies have pointed out that economically feasible algal production is of critical importance. Algal biomass after harvest can be used as forage, biogas feedstock or biofertilizer. In several review articles, it was shown that algal species were grown with satisfactory results on petrochemical effluent<sup>[119]</sup>, sewage wastewater<sup>[140]</sup>, piggery wastewater<sup>[121]</sup>, municipal wastewater<sup>[122]</sup>, domestic wastewater<sup>[123]</sup>, industrial wastewater<sup>[124]</sup>, aquaculture wastewater<sup>[78]</sup> and dairy effluent<sup>[74]</sup>. Barminski *et al.*<sup>[125]</sup> have provided a media recipes for raceway cultivation of N-fixing cyanobacteria by i) tank method, (ii) pit method, (iii) field method, and (iv) nursery cum algal production method. The former two methods are designed for small-scale and latter two are for bulk production on a commercial scale<sup>[55]</sup>.

## 6. Formulation of Algal Biofertilizers

Algal biofertilizer formulation has been developed and tested for commercialization. Among them, Dubey and Verma<sup>[126]</sup> used clay based inoculants for strain inoculation in soil for longer duration. The algae population in soil was about 10-70 times higher than that of the non-inoculated plots, even after four months<sup>[126]</sup>. Mishra and Pabbi<sup>[127]</sup> offered technology to farmers after getting a soil-based starter culture, which allowed them to produce the biofertilizer on their own with minimum additional inputs<sup>[127]</sup>. Tripathi *et al.*<sup>[128]</sup> used a so-called fly-ash approach, in which the cyanobacteria and nitrogen fertilizer were mixed to improve growth rate and yield of rice plants. This approach reduced nitrogen fertilizers demand<sup>[128]</sup>. In the study where cyanobacterial-based fertilizers were employed with two carriers (wheat straw and multani mitti-clay), the experiments were compared with traditional soil-based cyanobacterial biofertilizer. It was observed that both the straw-based and soil-based biofertilizer treatments have showed high yields when supplemented with 90 and 120 kg N/ha, respectively. It was thus proven that cyanobacterial biofertilizers can be formulated to maximize crop productivity and reduce inputs of chemical fertilizers in rice cultivation<sup>[129]</sup>. Paddy straw compost:vermiculite (1:1) as carrier-based formulation was also studied by Prasanna *et al.*<sup>[130]</sup> and Renuka *et al.*,<sup>[131]</sup> who found that the adaptation rates of some cyanobacteria (*Anabaena torulosa*, *Nostoc carneum*, *Nostoc piscinale*, *Anabaena doliolum*) were higher in the rice field when vermi-compost was used as their carrier<sup>[19]</sup>. The shelf-life of cyanobacterial biofertilizer can be augmented by selecting translucent packing material, dry mixing, and using paddy straw as a carrier. Dry mixing with a mixing ratio of 50:50 (carrier: cyanobacteria) has given better performance in inoculum loading and shelf-life<sup>[132]</sup>. Hori *et al.* used phytoextracts of neem (*Azadirachta indica*), bel (*Aegle marmelos*), and tobacco (*Nicotiana tabacum*) in controlling cyanobacterial disease during storage duration<sup>[133]</sup>. They found that tobacco waste was superior to others in disease prevention<sup>[132]</sup>. Moreover, akinetes were suggested to be used as a biofertilizer after drying processes, as they could be stored for at least several weeks when in a dried state<sup>[133]</sup>. It was found that algal growth largely depends on temperature and pH<sup>[59]</sup>. Cyanobacterial preservation can be obtained either by air drying or drying wet blue green algae in oven at 35-40 °C for 24 h in dark. This method is easy to be applied to ensure a high germination rate<sup>[134]</sup>. Foliar biofertilization by algae on willow monocultures was studied by Grzesik *et al.*

<sup>[70]</sup> and shown to significantly increase productivity. In particular, studies have indicated that the foliar application of *Acutodesmus dimorphus* aqueous extracts and formulation of growth media have created a positive effect on seed germination and plant growth. It was observed that *A. dimorphus* cellular extract and dry biomass could be used both as a biostimulant and a biofertilizer to trigger faster germination and to enhance plant growth and floral production in Roma tomato plants<sup>[68, 135]</sup>. Based on the above reports in the literature, we can conclude that the carrier-based formulation is more suitable for N<sub>2</sub> fixing fertilizer and soil conditioning, while foliar-based formulation is more appropriate for germination promoting effects.

## 7. Challenges and Measures for Commercialization

The commercial utilization of N<sub>2</sub>-fixing organisms in agriculture has encountered some difficulties. As Kumar<sup>[59]</sup> illustrated, factors such as a suitable carrier for individual algal, soil, and climate factors and biotic and abiotic stress in the field are the main constraints for commercial use of algae as biofertilizer. Cyanobacteria-based biofertilizers were widely used in paddy field cultivation due to their habitat and growth requirements. According to several studies, there are some limitations for cyanobacterial biofertilizers in areas possibly contaminated by pesticide residues, herbicides residues, and heavy metals (i.e., nickel and copper), or in land with high salinity. These factors can inhibit algal growth, cellular photosynthesis, and nitrogen fixing activity<sup>[136, 137]</sup>. He *et al.*<sup>[136]</sup> reported the inhibition on growth, synthesis of pigments, and photosystem II (PSII) activity of the nitrogen-fixing cyanobacterium *Nostoc* sp. by stress caused by the addition of butachlor. Debnath *et al.* studied the impacts of commonly used pesticide, fungicide, and insecticide on the growth and enzymes production of four cyanobacterial species-*Nostoc elliposporum*, *Scytonema simplex*, *Tolypothrix tenuis*, and *Westiellopsis prolifica*. It was observed that both the fungicides and insecticides at EC<sub>50</sub> concentration would cause an inhibitory effect on the expression of nitrogenase and glutamine synthetase in all four cyanobacterial species studied<sup>[138]</sup>. These four cyanobacterial strains have been the favored models for deeper understanding of intracellular metabolic processes involved in the production of compounds of medicinal and commercial value<sup>[109]</sup>. Sharma *et al.*<sup>[139]</sup> pointed out the need to adopt multidisciplinary approaches with a multiproduct process (biorefinery) strategy to harness the maximum benefit of cyanobacteria.

## 8. Conclusion

Algae-based biofertilizers have shown significant benefits in the development of green agriculture. Figure 2 illustrates future research areas for algae-based biofertilizers coupled with high energy generation. Beside N<sub>2</sub> fixation, they are able to increase soil fertility and give the PGPR effect to the crops. Some algal fertilizers can be produced as metabolic byproducts during the wastewater treatment processes, making them renewable sources for sustainable agriculture. Carrier systems for maintaining microalgal biomass for long periods of time are readily available from natural (soil, clay) and also renewable sources (paddy straw, multani mitti, etc.). Moreover, handing over the technology to farmers for their needs can create value and build up small-scale biofertilizer production within their individual circumstances. The development of organic farming without requiring a large

land for production or even effectively exploiting marginal land are other other advantages of algal fertilizers. In summary, applications of algal biofertilizers will meet the needs of sustainable agriculture with three main objectives: a healthy environment, economic profitability, and a socio-economic equity.

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### 10. Author contributions

Jainendra Pathak designed the study and wrote the manuscript, Jyoti Jaiswal, Ramesh K. Shukla, Deepak K. Singh helped in literature review. Rajeshwar P. Sinha reviewed and edited the manuscript.

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