Algae-Based Biofertilizers: A Biorefinery Approach

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Abstract

Current perturbations in the agrarian economies and the agro-environment have sparked concerns regarding the future food security and a dire need for sustainable agricultural practices without jeopardizing the environmental assets. One such major requirement for an agrarian society is a resilient nutrient source for agriculture. In this regard algal cells that are cosmopolitan in nature with unparalleled characteristics of high biomass productivity, high photosynthetic efficiency and ability to grow in barren and non-arable lands are attractive. They grow in a wide range of water systems especially the ones that are highly enriched

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with salts (saline) and nutrients (eutrophied) and in numerous contaminated and polluted systems as urban wastewaters. Apart from these primary benefits, the algal route also offers important byproducts that can be further used as value-added products in industries and as C neutral commodities that help to evade climate change by negating greenhouse gas emissions. The most important aspects are its efficiency as a biofertilizer that dynamically improves the soil health and its physicochemical behaviour. The wastewater-grown algal microflora is exceptional in imparting the appropriate mineral nutrient mix with essential vitamins and plant growth promoters together with increasing the water holding capacity of the soil. Algal communities from wastewaters with optimal NPK ratio and secondary nutrients are therefore model biofertilizers. They can be substitutes of the conventional chemical fertilizers due to its ubiquity, enhanced metabolic flux, short generation time and inherent capabilities to transform inert N into plant-available N (N fixation). All the above-mentioned characteristics, techno-economic feasibility and environmental benefits make algae the most beneficial and demanding bioresource of the twenty-first century.

Keywords
Biofertilizer · Algae · *Spirulina* · Wastewater · Nitrogen · Nutrients

10.1 Introduction

Agricultural food security is essential for development and globalization. Existing global population is 7.5 billion and is expected to reach ~10 billion by 2050. Ensuring adequate food supply globally would require an annual production of cereal grains by 50% that indicates an increase from 2 to 3 billion tons/annum. This mammoth goal presses serious concerns on improving present-day strategies and developing technologies to increase the agricultural productivity and thus exerts a great burden on the present agricultural sector to accomplish the towering food demands. This giant upsurge in anticipated food production can be only possible by

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either (a) increasing the area under cultivation or (b) increasing the efficiency/productivity of the existing cultivable lands. The possibility of procuring more land and reclamations into agricultural lands is presently rampant (Ramachandra et al. 2009, 2013) and has higher environmental risks and externalities (Ramachandra et al. 2015). Higher land use and land cover changes have altered the physical, chemical and biological integrity of the soil (pedosphere) and have rendered them infertile. Thus, there are low possibilities of the first option although presently there are a lot of research on salt-tolerant species possibly grown in saline soils, but such research are still within laboratories and are presently under progress that needs more field trails and analysis. However, the second option though challenging is in fact practical, and various research outcomes show revealing results in improvement of soil fertility and agricultural productivity using environmental-friendly irrigation and agricultural processes that ensures food security sustainably.

Conventional cropping practices are presently overdependent on man-made fertilizers, weedicides and insecticides, tilling practices and intensive irrigation, which have surged a reasonably high quantum of food grains across the last five decades. This rapid surge in production of food grains termed as green revolution, although has created surplus food grains in developing nations, but has intensely costed the quality of the environmental assets and raised concerns for human health and hygiene. Very apparent among them are the air, water and soil pollution, loss in soil fertility, alterations in soil property, excessive use of natural resources, increased nutrient enrichments and accumulation with consequent algal blooms, and moreover all this has added to increased cost in agricultural production. Thus, it becomes a challenge to identify resilient solutions to address these problems in figuring out sustainable nutrition sources, efficient, less-energy and resource-intensive agricultural practices to cope up with the towering food and resource demands in the future. Integrating the traditional agricultural practices like crop mulching, transplantation and crop rotation with ubiquitously found algal biomass can serve as a sustainable solution for higher agricultural productivity with no environmental externalities. Traditional agricultural practices were driven mostly by naturally fixed nutrient sources as green plant-based fertilizers and organic manures and using local water resources as man-made lakes and ponds with efficient bio-based yard manures that taps nutrients from domestic wastewaters. Due to globalization and higher concentrations of population in urban conglomerates, the per capita land footprint has drastically reduced that lacks places like yard and gardens where these nutrient-laden water could be used. Similarly, in periurban and rural setups, the soil qualities have been drastically deteriorated with overuse of chemical fertilizers and other harmful chemical inputs like pesticides and weedicides. Various routes of production of nitrogen (N)- and phosphorus (P)-based chemical fertilizers are depicted in Fig. 10.1. This has not only affected the food production but has also compromised with the environmental quality of the agri-sectors. Use of appropriate agricultural tools and approaches is the only way to revive and rejuvenate rapidly lost agri-landscapes and nutrient-rich soils (Mason 2003). The principles of sustainability and ancient agricultural philosophy hinge around eco-friendly methods and cost-effective cultivation strategies with the aid of native soil microflora. This further strengthens the views on

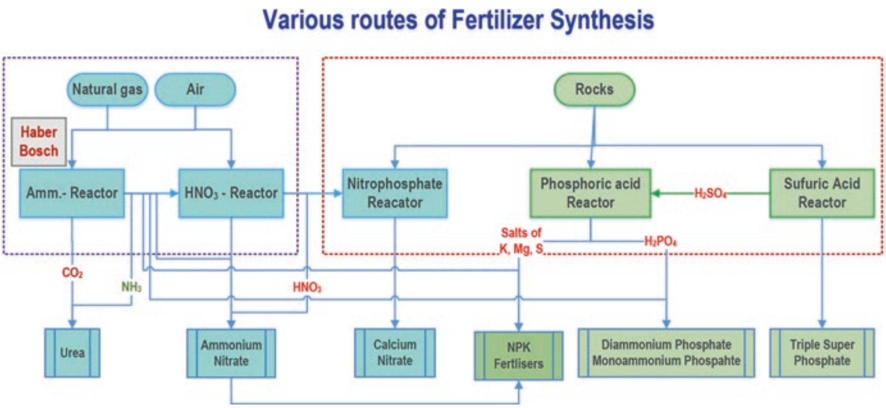

Fig. 10.1 Schematic representation of commercial synthesis of N- and P-based fertilizers

using natural resources optimally and usage of ecological bioprocesses that makes sure that there are no waste components left following a biorefinery approach. This essentially closes the nutrient loops within the system and at the same time increases the crop yield and agricultural productivity.

Soil microflora have been known to increase the soil fertility and help in higher biomass productivities (Koller et al. 2012). However, the algal microflora has been also realized as a true bio-based fertilizer for eco-friendly and pollution-free agricultural practices (Yamamguchi 1997; Benson et al. 2014; Mahapatra et al. 2015). Considering the fact that algal biomass are key to crucial ecosystem processes, they can play a pivotal role in improving crop yield and at the same time mitigation greenhouse gas (GHG) emissions (Singh 2011; Mahapatra et al. 2015). Studies conducted by Singh have reported essential roles of blue-green algae (BGA) in ecological restoration of degraded lands (Singh 2014). These algae although photosynthetic are adaptive to extremes of conditions and are known to survive at critical light intensities with minimal nutrients as C and N levels with a bare minimum water requirement (Castenholz 2001). Being efficient N-fixating agents, they are highly independent of perturbations in terrestrial N pools that produce key essential amino acids, growth promoters and hormones that are essential for a good growth in cropping systems (Pandey et al. 2004). Algal assemblages can derive most of their necessary nutrients from wastewaters, with capacities of breaking down highly complex, toxic and xenobiotic compounds (Cohen 2006).

Optimal plant growth and agricultural productivity are directly dependent on nutrient availability in balanced quantities (Chen 2006). In the developing world, the agricultural yield is dependent on nature and the type of soil, as most of the farmers belonging to the poorer section of the society cannot afford synthetic agricultural fertilizer unless it is subsidized. The lost fertility of the agricultural soils can be restored by usage of improved crop varieties and efficient nutrient management practices. This essentially suggests use of the abundantly present natural bioresources for increasing the nutrient status of the crops and allowing more potential biological nutrient transfer methods. In such cases, biofertilizers play a major role and are essential components of the controlled mineralization and fertilization process. Being cost-effective, eco-friendly and a renewable resource, this can altogether replace or augment the presently used cost- and energy-intensive chemical fertilizers. These are primarily live and/or dead cells with beneficial organisms applied to plant and soil systems. They rapidly colonize the rhizosphere and thus enhance plant growth and development, thereby converting unavailable mineral forms into essential nutrients via processes as N fixation, mineralization and solubilization of rock phosphates (Rokhzadi et al. 2008). These organisms eventually become a stable part of the root zone section that accelerates the agricultural yields and also safeguards the agri-systems from myriads of pests and pathogens (El-yazeid et al. 2007). There have been a number of studies and reviews on these beneficial aspects of the microorganism as biofertilizer (Wani et al. 1995; Yamaguchi 1997; Rai et al. 2000; Koler et al. 2012; Geisseler and Scow 2014).

10.2 Conventional Biofertilizers to Algae-Based Fertilizers: Definition and Scope

Biofertilizers can be defined as a composite of live or latent organisms with characteristic ability to fix atmospheric nitrogen, solubilize mineral phosphates or degrade organic matter that targets soil and composting regions to increase the abundance of a beneficial microflora that accelerates key nutrient mineralization and uptake processes to augment the quantum of nutrients in the medium. Biofertilizer terminology comprises of every organic resource for cultivation that is concentrated in available form, absorbed by the plants through microbes and plant association (Singh 2015). Traditionally composting and using the compostable materials (food materials, cow dung, agri-residues) mark the initiation of use of organic matter as plant nutrients that has been well known to the ancestral agricultural communities and has been passed on as heritage to the modern community. The realization of the true potential of these applications is the modern-day biofertilizers that accelerate the decomposition kinetics of the organic residues and agricultural byproducts (Singh 2014). In Southeast Asia that is the primary agrarian belt, industrial production of these beneficial microbes has been in progress. There are a number of reports on efficacy of algal community mostly cyanobacterial biofertilizers on crop productivity. One of the very widely used biofertilizers that has been often used for N fixation and assimilation into plants is the *Rhizobium* in legumes. On the other hand, the applications of mycorrhiza and *Azospirillum* are also studied to a great deal. It has been reported that the mycorrhiza inoculums have been rapidly adapted to the tropical Southeast Asian conditions as in India and Malaysia and are being actively used in agricultural industry. This encourages commercial production of biofertilizers that enhances soil fertility and ameliorates toxic effects of soil, checks growth of soil pests and restricts diseases with improved water and mineral absorption. Moreover, these microbial assemblages can be grown easily in substrates as mine sands and agricultural wastes, making them economically viable with a lower environmental footprint. However, biofertilizers are reported to be expensive than chemical fertilizers as it requires a great deal of skill for bioconversion of waste substrates and production of high-density inoculum. Furthermore, the tests on the land applications have shown that the activity of biofertilizers in terms of fertilization efficiency is slow compared to chemical fertilizers. During its production, great attention and care are taken for homogenizing, mixing and storage with the dried inoculum forms as powders to ensure viability of the inoculums for an extended use. The conventional biofertilizers as mentioned earlier deal with living microbes; therefore the growth activity and nutrient transformations also greatly depend on the surrounding environment and thus can have high inconsistencies in their activities. On the other hand, the presently reported algal biofertilizers are not based on the active algal biomass but are high-nutrient-enriched stable biomass mix that have active growth stimulants with nourishing vitamins, microelements and hormones that have been showing promising results. The algal biomass can itself act as both nutrient and carrier materials for biofertilizers, as it has a better shelf life (post drying) that is less thermo-sensitive and is robust for storage and transportation, making it a better choice as a biofertilizer.

Algal growth enhances the growth of rhizosphere bacteria through beneficial exudates and exopolysaccharides (Kapustka and DuBois 1987) that become the carbon (C) source for the soil bacteria. When the dried forms of algae are applied to the crop soils, after mineralization, the cell extracts from BGA and diatoms impart stimulatory effects on the growth of bacterium that produces siderophore (Amin et al. 2009). The mechanism involves the photolysis of Fe siderophore that triggers Fe assimilation in moist conditions eventually promoting the association of algae extracts and bacteria, where the organics released by algal members are used as food for bacterial members. This helps in bioavailability and assimilation of Fe through Fe chelators like siderophores under low Fe and N conditions that happens mostly in aquatic environments or under waterlogged conditions. Besides this BGA plays a major role in transformation of soil micronutrients (Fe and Mn) together with production of organic acids that have key chelating abilities that enhances the bioavailability of micronutrients for the growing plants. Algal species as *Anabaena flos-aquae*, *A. cylindrica*, *Synechococcus leopoliensis* and *Anacystis nidulans* have shown to excrete strong copper-complexing agents ($^{c}K > 10^{8}$), while eukaryotic algae have been only found to release relatively weak copper-complexing agents ($^{c}K < 10^{7.5}$) (McKnight and Morel 1980). Although these findings were mostly reported from studies in aquatic systems, the results provide a huge scope for further studies in an agricultural setup to understand micronutrient dynamics involving siderophore productions and connections between the soil algal and the plant species in the biofertilizer activity for soil nutrient translocation.

10.2.1 Production of Biofertilizers: The Algal Industry

The commercial production of biofertilizers considers several aspects as the microorganism's growth and nutrient profile, the assembly of the microbes, suitable conditions of maintaining an active biomass and formulation of the inoculum.

Box 10.1: Steps in Commercial Production of Biofertilizers

1. Exploration and identification of active microbial consortia
2. Isolation of select active organisms
3. Rapid screening and selection of beneficial target microbes
4. Efficacy of microbial target population
5. Selection of carrier material and the methods
6. Selection for the method of propagation
7. Investigations on nutrient studies and design of porotype and testing
8. Field trials and large-scale testing

For commercialization, the key emphasis is on inoculate formulation, nature of application and packaging and storage of products (Box 10.1).

The very first step in realizing the objective of the project is to identify what kind of organisms is required for the study. It is therefore crucial to check whether it is an active mineralizing bacteria or oxidative bacterial communities for conversion of organic acids or phosphorus-binding/phosphorus-solubilizing bacteria or N-fixing bacteria or a combination of them. According to the intended function of the microbial community, the microbial assemblage is explored and isolated, mostly from soil matrices, plant roots, marshes, water bodies, etc. This is followed by the routine laboratory-based culturing techniques where they are grown as plate cultures to flask-based cultures, and finally the best assemblages are selected. The bulk of the biofertilizer comprises of the carrier material and thus requires a rigorous selection of materials suitable for the purpose. The carrier material selection is again based on the final form of the biofertilizers, e.g. powder or liquid. In case of production of powdered biofertilizers, peat and tapioca flour have been used extensively. It is necessary to formulate the simplest and easiest ways to propagate the organism that are based on the experimental finding on environmental conditions and the growth suitability in various growth setups. The identification of the optimal propagation methods aids in finding out the best conditions for the organisms to grow. Prototype development and testing are carried out after testing the suitability of growth medium and the carrier material. During the final phase, the biofertilizers developed are tested on large-scale setup to understand the efficacy and limitations to various field conditions. After the development of the biofertilizers, it is necessary to ensure prolonged storage and avoid degradation and possible contamination by other types of bacteria for the reason which the carrier materials are sterilized with either irradiation with UV/gamma rays or autoclaving the materials. For these reasons, carrier material selection becomes the most crucial step for the design and development of the biofertilizers. Virtuous carrier material should be inexpensive and must be available in adequate quantities. At the same time, it should be nontoxic to the plants and must not have any side effects in a long run. As characteristics of appropriate carrier materials, the ideal material must have great moisture holding capacity and nice adhesion abilities to bind to seeds. Along with this, the carrier materials must have a good pH buffering ability, easy processing and sterilization with simple comminution.

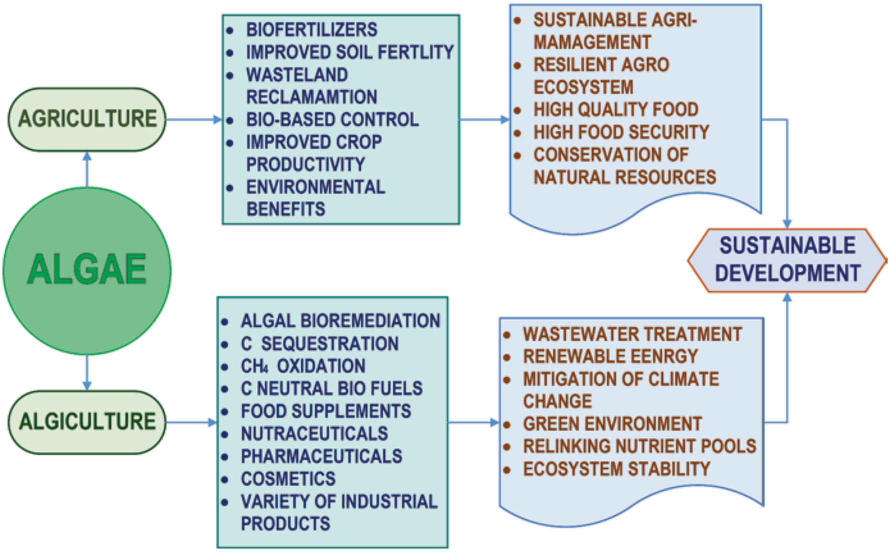

Fig. 10.2 Advantages and characteristic features of algae used as biofertilizers

Wastewater is being increasingly used to grow the algal assemblages rapidly that have immense potentials to immobilize nutrients and make the nutrients bioavailable later for a rapid growth of crops through controlled release of nutrients. The algal species can be grown together with the plants that helps in N fixation and balancing nutrient imbalances or can be used in the dried form to augment the fertility of the land with essential nutrients with an optimal ratio of NPK. A multi-tier algal cropping (algiculture) is envisioned that comprises of various algal communities as a function of light penetration and depth in the agricultural crops especially rice cultivation, which has standing waters during its growth and development (Mahapatra 2015). The various advantages of algal use in (a) agriculture and as (b) algiculture for sustainable and green future have been depicted in a schematic diagram in Fig. 10.2.

10.2.2 Microbial Assemblages as Biofertilizers

The most important class of biofertilizers includes (a) nitrogen fixers, (b) phosphorus solubilizers and (c) potassium solubilizers with a combination of various other organisms for symbiotic association or support as fungus and moulds. The bacterial assemblages or isolates used as biofertilizers have their association with plant roots. The most popular N-fixing bacteria *Rhizobium* has a symbiotic association with the root nodules of leguminous plants. *Rhizobacteria* on the other hand are found on the root surfaces and rhizosphere. As phosphorus is a limiting nutrient in any kind of

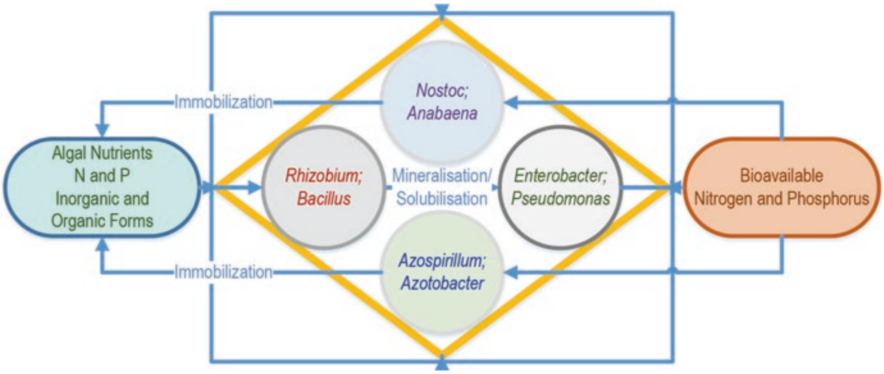

Fig. 10.3 Mineralization pathways of nutrients in algal-bacterial association

Table 10.1 Functions of major microbial class of biofertilizers with various species

Sl. no.	Microbial biofertilization	Microbial species
1	*Free-living N-fixing bacteria*	Obligate anaerobes: *Clostridium pasteurianum*
		Obligate aerobes: *Azotobacter*
		Facultative anaerobes
		Photosynthetic bacteria (*Rhodobacter*)
		Cyanobacteria
		Methanogens: *Methanococcus*
2	*P solubilizer*	*Bacillus megaterium*, *Bacillus circulans*, *Bacillus subtilis* and *Pseudomonas striata*
3	*K solubilizer*	*Bacillus mucilaginous*

ecosystem. The P-mineralizing bacteria and fungi mineralize the insoluble and unavailable phosphorus and make it bioavailable to the plants (Gupta 2004; Mahapatra 2015). This happens due to solubilizing the bound P in soil systems and organic matter by organic acid productions in bacteria that helps in lowering the soil pH and triggers release of the bound P forms. Many of the biofertilizers are targeted for specific type of the crop plants as *Rhizobium* for legumes, cyanobacteria for grains (rice and wheat) and *Azolla* for pulses and cereals. Many other categories of biofertilizers as vesicular arbuscular mycorrhiza (VAM), phosphorus-solubilizing bacteria (PSB), *Azospirillum* and *Azotobacter* can be considered for a wide range of crops and cultivars. The mineralization and solubilization routes across the algal-bacterial association are depicted in Fig. 10.3. These biofertilizers are associated with majority of the crops and thus enhance plant growth to suitable nutrient capture. In many cases, these microbes in association with the plant roots and soil systems ensure inhibition of pests and crop-damaging bacterial communities. Some of the extensively used biofertilizers are illustrated in Table 10.1.

10.3 Wastewater Algae as a Suitable Source of Biofertilizers

10.3.1 Algal Growth, Bioproducts and Nutrient Analysis from Urban Wastewaters: Case Study

Algal research has gained special impetus and scope in the present era. The exploitation of the algal whole cell biomass for extraction of value-added products in tandem with wastewater treatment ensures its techno-economic feasibility with no environmental externalities. A major area of interest in algal biotechnology is the economic benefits associated with the mass culture and biomass use of algal species thriving in tropical wastewaters. Most of the wastewater generated in the developing world remains unattended and enriches the surface and the ground waters with higher loads of nutrients rendering it unsuitable for any use (Mahapatra et al. 2011a, b, c; Mahapatra et al. 2017). The algal species can be used as robust treatment systems to recover nutrients from their wastewater and municipal landfill leachate (Naveen et al. 2016) by harvesting whole cell algal biomass (Mahapatra and Ramachandra 2013). The algal biomass have shown potential to remove nutrients as C, N and P as unialgal cultures or as microbial consortia with the production of valorizable algal biomass that shows significant quantity of lipids (Mahapatra and Ramachandra 2013; Mahapatra et al. 2013a, 2014; Ramachandra et al. 2015). Large-scale algae-based treatment systems have been also studied and have shown a higher techno-economic feasibility (Chanakya et al. 2012,2013) for algal single-cell proteins (SCP) (Mahapatra et al. 2013b, 2015) with a lower environmental impact (Ramachandra and Mahapatra 2015; Ramachandra et al. 2015). The algal modules designed at the Indian Institute of Science (IISc), Bangalore, have been successfully employed for nutrient (C, N and P) capture and recovery at the Jakkur Lake in Bangalore City and show promising augmentation to the conventional treatment, providing purified water that can be recycled and reused (Ramachandra et al. 2014; Mahapatra 2015).

In the present study, *Spirulina* species, predominant in urban wastewater-fed systems, have been investigated for their role in wastewater bioremediation and as nutrient sources for land applications as biofertilizers. *Spirulina* belong to blue-green algae (cyanobacteria) and are mostly spiral shaped in filaments comprising of multicellular trichrome arranged in an open helix. *Spirulina* sp. was isolated from the wastewater-fed lakes in Bangalore City, cultured in synthetic wastewater media (Mahapatra et al. 2013a) and then grown in concentrated and settled urban wastewaters (Mahapatra et al. 2013a). Various nutrient analyses were carried out by following standard protocols (APHA 2005). The macromolecular compound analysis for lipids, carbohydrates and proteins was performed by following standard protocols (Mahapatra 2015). Outdoor rooftop batch studies (10 litres) were performed in polypropylene open bioreactors (Mahapatra et al. 2014). The culture was maintained for 10 days under natural illumination at temperature ranging from 27 to 32 °C. Manual agitation was provided thrice a day. For algal and nutrient quantification, 200 ml of water was sampled every 2 days and was replaced with deionized water. Care was also taken to compensate the water loss due to evaporation. The algal biomass was

Table 10.2 Nutrient removal and treatment efficiency by algal cultivation

Sl. No	Treatment parameter	Initial concentration (mg/l)	Final concentration (mg/l)	Treatment efficiency (% removal)
1	*COD*	680 ± 12	28 ± 2.4	95.88
2	*TN*	96 ± 7.2	3.8 ± 0.35	96.04
3	*TP*	35 ± 4.3	2.2 ± 0.26	93.71
4	*Ortho-P*	22.5 ± 1.77	1.85 ± 0.28	91.78
5	*Amm.-N*	77 ± 4.78	1.04 ± 0.33	98.65
6	*Nitrate-N*	1.44 ± 0.26	1.3 ± 0.38	9.72
7	*Nitrite-N*	0.06	U.D.	–

tested for growth, nutrients and macromolecules. The cell morphologies and growth were checked with light and electron microscopy as per the protocols used in earlier studies (Mahapatra et al. 2014). The growth was quantified by both spectrophotometry and gravimetry.

10.3.2 Wastewater Treatment with Nutrient Recovery in Algal Biomass

The results for the cultivation studies showed thick growth of *Spirulina* spirals entangled with each other and were mostly concentrated on the upper layer of the reactors. Being large cells with very frequent entangling, the *Spirulina* biomass was quantified by spectrophotometry and was verified for incremental growth with gravimetry. Higher growth was observed at the end of the eighth day during the cultivation period (specific growth rate, 0.35/day) with a maximum biomass density of 1.04 g/l. The biomass productivity was 130 mg/l/d, and during the experiments, the pH varied from 7.66 to 8.16.

The growth experiments showed near complete C, N and P removal illustrated in Table 10.2. As high as 95.88% of COD was removed indicating mixotrophic intake of organic C in *Spirulina* species that proves its suitability for treatment of untreated wastewater. The COD dropped from 680 to <30 mg/l, highlighting positive prospects of *Spirulina* sp. for treatment of municipal wastewaters. Similarly, the TN dropped from 96 to <4 mg/l (~96% removal) and TP dropped from 35 to 2.2 mg/l (~94% removal) indicating its ability to recover essential inorganic nutrients. There were no significant changes in the nitrate-N concentration that were found, mostly consistent across the experiment, i.e. from 2.02 mg/l (initial concentration) to 1.72 mg/l (final concentration). The nitrites were mostly undetectable (UD). However, ammoniacal nitrogen (amm.-N) concentrations were greatly removed from 77 to 1.04 mg/l (~99%) that is suitable for aquaculture and thus shows enormous abilities to capture wastewater nitrogen. Moreover, the reactive phosphorus (ortho-P) was also significantly reduced from 22.5 to 1.85 mg/l (~92% removal). Being a non-heterocystous BGA, *Spirulina* species assimilates high N from the wastewaters that favours its growth and synthesis of valued proteins in the cells.

Table 10.3 Macromolecular and biochemical composition of algal biomass and fertilizers

Macromolecular composition	*Spirulina* sp. (cultivated)	*Spirulina* sp. (wastewater-fed lakes)	Organic fertilizer	Chemical fertilizer
Lipids (%)	11	9.8	–	–
Carbohydrate (%)	22	18.2	–	–
Protein (%)	48.125	45	–	–
Nitrogen (%)	7.7	7.2	1.09	12.4
Phosphorus (%)	0.88	0.71	0.7	6.6
Potassium (%)	1.76	2.44	1.27	12.5
Calcium (%)	0.67	0.52	0.48	0.1

The macromolecular composition of the algal biomass cultivated as rooftop cultures and collected from *Spirulina* sp. blooms in urban lakes is illustrated in Table 10.3. This has been compared with the organic fertilizers and chemical fertilizers available in the market. The results show higher protein quantities (~48%) due to high N content (7.7%) in present outdoor cultures compared to surface waters attributed to the difference in nutrient loads. Besides this, the cultivated *Spirulina* sp. showed relatively high lipids and carbohydrate contents as compared to the species collected directly from the lakes. This is possibly due to availability of high organic C in addition to inorganic nutrients in the wastewater feed. However, the N content of the cultivated algal species was almost 62% of that in commercially available chemical fertilizer. Similarly, lower levels of P (13.3%) and K (14%) were recorded in the cultivated algal species. However the Ca content in the *Spirulina* species cultivated was much higher (~6.7 folds) than chemical fertilizer. Moreover, the cultured algal species grown in wastewater nutrient content were far better than the organic fertilizers (Table 10.3). Albeit having a lower percentage of NPK compared to chemical fertilizers, the algal biofertilizers from wastewaters have shown to enhance plant growth to a similar extent as chemical fertilizers due to higher amounts of secondary and trace nutrients that are crucial and central in plant physiology and metabolism. This shows immense scope of facilitating *Spirulina* sp. cultivation in urban wastewaters for wastewater treatment and nutrient recovery as whole cell algal biomass that is suitable as biofertilizer. In this context, further potting experiments with wastewater-grown algal species are being carried out to check the efficacy of algal application as dried whole cell biomass as a biofertilizer for improvement in soil nutrients with plant growth and development.

10.3.3 Effectiveness of Algal Biofertilizers (*Spirulina* sp.) Grown in Wastewaters with a Biorefinery Approach: Scope for Further Research and Development

Table 10.3 shows the biochemical constitution of the wastewater-cultivated *Spirulina* species and its usefulness as a biofertilizer. There are many studies that have reported higher plant growth with soil enriched with algal input, i.e. *Spirulina* biomass, showing increased plant height (red Bayam, arugula and pak choy) in addition to greater fresh/dry weight when compared to controls without *Spirulina* amendment (Wuang et al. 2016). However, there were no apparent differences in root lengths, foliar number and overall chlorophyll content (Tripathi et al. 2008; Saadatnia and Riahi 2009; Wuang et al. 2016). The comparison of the nutrient value of the dried algal biomass after cultivation with the commercial fertilizer and organic fertilizer showed significant differences and indicates that the whole cell algal biomass can itself act as both a carrier and bio-stimulant (nutrients) for effective fertilization of crops. Studies conducted on growth of plants with algal biofertilizer (*Spirulina* sp.) and chemical fertilizer showed no significant difference in growth and development. However, the net biomass was high in case of chemical fertilizers. Therefore, such algal biomass can act as slow/passive fertilizers or as essential supplement to the chemical fertilizers to augment beneficial micronutrients that aid development of crop plants (Rai et al. 2000). Studies on wheat cultivation using wastewater-grown algal assemblage showed higher growth and yield with ~53% increase in leaf chlorophyll compared to controls (Renuka et al. 2017) and improved the nutritional status of plants (Prasanna et al. 2013). Moreover, algal biomass inputs as biofertilizers obtained from wastewaters aided in slow release of nutrients in a cropping study involving tomatoes that have also shown improved quality of the crop with high sugar and carotenoids. Improved dry weight through applications of green algae *Acutodesmus dimorphus* were observed together with enhanced germination rate, growth and floral production in tomato plant (Garcia-Gonzalez and Sommerfeld, 2015). Experiments on algal biofertilizer applications to soils also reveal production of growth-promoting compounds essentially comprising of plant growth hormones that profoundly help in growth and maintaining good plant health (Karthikeyan et al. 2007; Perez-Montano et al. 2014). More investigations on understanding the dynamics of nutrient translocation through wastewater-grown algae (various wastewater sources) and at various scales (plot trials to field scale) and its evaluation can aid in involving algal biomass as essential fertilization strategies for optimal nutrient management in tropical agricultural setups.

Rampant and imbalanced use of chemical fertilizers without proper scientific know-how of its dose response, effects and implications in land with reduced usage of agricultural crop residues has highly deteriorated the soil nutrient status globally. This has led to deficiency in the macro- and micronutrients, thus impairing the geomorphology, the soil chemistry and the biophysical conditions of the soil (Geisseler and Scow 2014; Murase et al. 2015; Li et al. 2017). Municipal wastewater (urban sewage) can be used as a suitable nutrient source for fertilization of nutrient-deficient soils for agriculture. However, wastewater comprises of a variety of pollutants as heavy metals and pathogenic microorganisms that restricts its direct use in agriculture. An ideal and scientific way for management of untreated nutrient reservoirs as urban wastewaters is by the use of algal communities proliferating in such wastewaters to (a) recover nutrients immobilized as algal whole cell biomass (Mahapatra 2015) and (b) at the same time aid in bioremediation of wastewater (Mahapatra et al. 2014) with (c) reducing GHG emissions (Ramachandra and Mahapatra 2015). Cultivating algal microflora in wastewaters and usage of algal biomass not only enhance the soil nutrient status but also help in providing growth-promoting triggers/compounds and restrict the growth of pathogenic undesirable bacterial communities due to an alkaline environment, through oxygenated conditions (Chaudhary et al. 2012; Prasanna et al. 2013).

Studies on biofortification of wheat by algal application (BGA) aid in improving soil fertility, improved crop yields and also fortify essential trace nutrients in plant tissues and whole grains (Rai et al. 2000; Renuka et al. 2016; Adak et al. 2016). It has been shown that application of algae grown in municipal wastewaters provided ~25% N savings during wheat productions (Renuka et al. 2016). Wastewater-grown algal species have increased the bioavailability to trace nutrients as Cu, Fe and Zn in soils. The N metabolism and fixation are directly influenced by the assimilation and availability of essential catalysts and cofactors as Zn and Fe. Counteractively, the expression of the transporters for Zn and Fe is also dependent on N status in the plant cells (Guo et al. 2014). Studies have shown 25–50% N replacement with cyanobacterial amendment (Prassana et al. 2013) and ~75% N replacement (Renuka et al. 2017) in wheat species. The applications of chemical fertilizers have shown lowest soil chlorophyll content that indicates harmful impacts on soil algal photosynthetic systems, and at the same time algal addition has improved chlorophyll content and N fixation in soil systems. Soil organic carbon (SOC) is an essential indicator of the soil status, health and suitability for agriculture or any cropping system (Lal 2004; Sa et al. 2017). Algal consortia amended to such systems with low organic carbon soils showed ~50% high SOC as compared to control.

Dehydrogenase enzyme activity is considered as one of the most crucial indicators in total soil microbial action effectiveness and plays a major role in organic matter bio-oxidation (Zhang et al. 2010; Leon et al. 2017). This enzyme activity is used as a significant index for soil fertility and health due to its impacts on the physicochemical and biochemical properties of the soil. Studies conducted on biofertilizer application on soil systems have shown a higher dehydrogenase activity with a strong correlation on soil organic carbon. But, during waterlogged condition and prevalence of anaerobic environment, there can be a very high dehydrogenase activity that impacts the transport and availability of Fe to the plants due to its utilization as Fe(III) as terminal electron acceptor (Das and Varma 2010).

Previous studies have shown dried BGA as soil inoculants that enhances the fertility of crops and rice cultivars (Watanabe and Konishi 1951; Venkataraman and Neelakantan 1967; Mishra and Pabbi 2004). These studies have shown increase in the rice grain yields (~20%) in agricultural field-based experiments. Studies on seed germination (seed viability) of BGA members as *Spirulina* sp. can be indicators of the performance of biofertilizers in the field. Studies conducted by rinsing and soaking the rice grains with BGA culture showed more effectiveness against sulphate-reducing mechanism and thus aid in rapid germination and faster growth of the rice seedlings (Nanda et al. 1991; Shariatmadari et al. 2011; Dineshkumar et al. 2017). Moreover, studies conducted on seed germination by Wuang et al. on three vegetable cultivars as Chinese cabbage, kai lan and white crown showed a threefold increase in the dry weight of the germinated seedling compared to the control with no algal inputs (Wuang et al. 2016). The key findings of the study showed *Spirulina* biofertilizers as seed germination enhancer, and their activities vary from species to

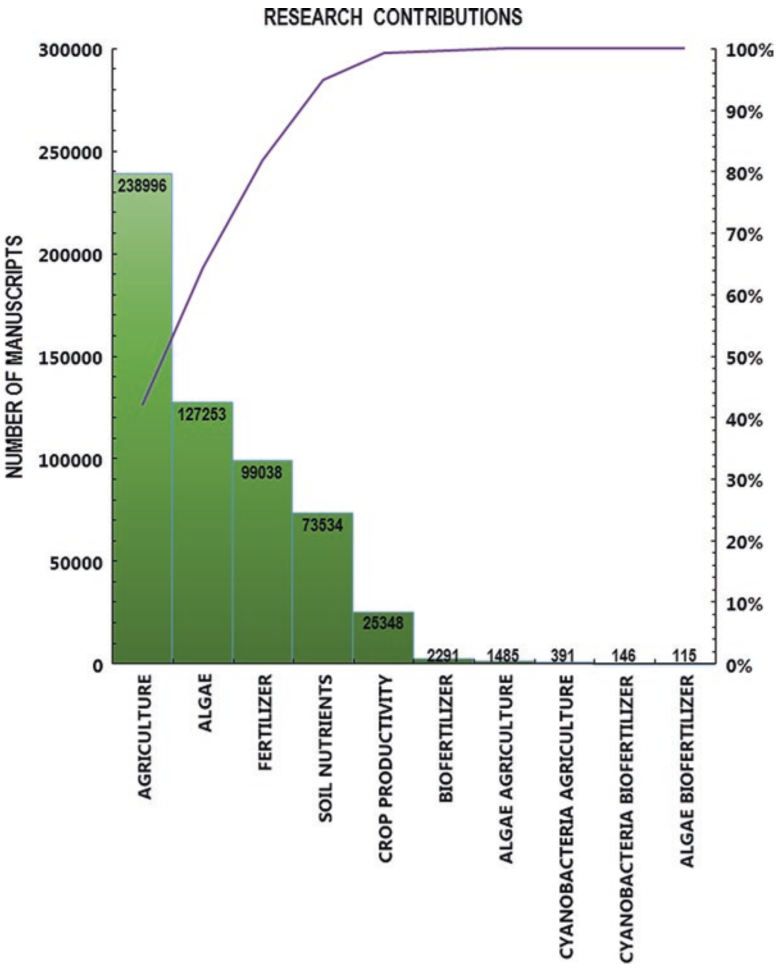

Fig. 10.4 Research contributions from agriculture to algal biofertilizer in the last 10 years

species. *Spirulina* amendment to soils growing pulses as green gram showed higher productivity (Aung 2011). Previous investigations on biofertilization have shown enhanced seed germination rates for sunflower (Jalaluddin and Hamid 2011); dill (Miri et al. 2013); and cumin, marigold and tomato (Garcia-Gonzalez and Sommerfeld 2015). Other studies have also reported higher growth enhancements in maize plants through biofertilization of red algae (Safinaz and Ragaa 2013). Besides BGA studies on green algae as *Chlorella vulgaris* showed higher plant development in lettuce (Faheed and Fattah 2008).

A review on the total number of peer-reviewed manuscripts published in the last 10 years based on Scopus and Thomson Reuters Indices on miscellaneous aspects of agriculture showed around ~2.4 lakh articles as illustrated in the Pareto chart (Fig. 10.4). However, the total articles on various aspects of algae, e.g. biotechnology, bioproducts, bioresources, engineering, nanosciences, chemical biology, biophysics, etc., were ~1.2 lakhs, i.e. almost half that of the manuscripts on various aspects of agriculture. The research on fertilizers mostly emphasizing crop studies, fertilizer mining, extraction and production, fertilizer amendments and economics and metallurgical engineering were around ~1 lakh. Articles on scientific findings in (a) soil sciences and nutrients, (b) crop productivity and (c) biofertilizers were ~73, 25 and 2 thousands, respectively. Nevertheless, studies on (a) algal use in agriculture, (b) BGA-based agriculture and c) BGA-based biofertilizer compared to the earlier figures were only 1485, 391 and 146, respectively. More importantly, very few studies, i.e. 115, have been conducted on the frontiers of algal biomass usage as nutrient-rich sources for biofertilizers mostly for land applications in agricultural setups.

Although these numbers might be indicative, there is definitely a huge scope for research in the field of algal biofertilizers in the context of increasing instances of climate change, decreasing soil organic matter and nutrients and unsustainable extraction and industrial fixing of inorganic nutrients as chemical fertilizers. In this context, the present study shows prospects of algal species as *Spirulina* as a byproduct of wastewater treatment that has potentially high nutrient values with optimal secondary and trace nutrient concentration suitable for land applications as biofertilizers. Wastewater treatment, nutrient recovery through sustainable algal bioprocesses and algal biomass application in agricultural fields demonstrate a biorefinery approach suitable for tropical agrarian economies to reach a zero-waste agricultural society that nurtures sustainable development.

10.4 Conclusion

The present communication shows the future prospects of biofertilizers in the agrarian economy and highlights the potential of wastewater-grown algae for enhancement of nutrient availability to plant systems. Cultivation of *Spirulina* sp. isolated from urban wastewater-fed lakes in outdoor rooftop batch cultures with concentrated wastewaters showed near-complete removal and recovery of essential macronutrients as C, N and P. *Spirulina* sp. was efficient in completely transforming the labile N and P forms as amm.-N and ortho-P into algal biomass and showed a mixotrophic growth evident from significant COD removal during the culturing experiments. The macromolecular composition revealed considerably high protein content useful for usage of whole cell algal biomass directly as N-rich biofertilizers. The macro-elemental composition showed significantly high NPK content better than available organic fertilizers and relatively low nutrient content when compared to chemical fertilizers. Despite this, a high micronutrient content as Ca in wastewater-grown algal biomass provides additional advantage for algal biomass to be used as biofertilizer. This enhances the soil macro- and micronutrient content and augments plant growth, for a sustainable and green agricultural future with a better food quality, realizing the biorefinery approach with a zero-waste concept.

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