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Chapter 40

Microalgae and cyanobacteria role in sustainable agriculture: From wastewater treatment to bio-fertilizer production.

Giovanni Antonio Lutzu^{1*}, Ali Parsaeimehr², Gulnihal Ozbay², Adriana Ciurli³, Leonardo Bacci³, Ranga Rao Ambati⁴, Ravinshankar Gokare A⁵, Alessandro Concas^{6,7}

¹Teregroup Srl, via David Livingstone 37, 41122 Modena, MO, Italy

²Department of Agriculture and Natural Resources, College of Agriculture, Science and Technology, Delaware State University, Dover, DE, 19901, USA

³Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, I-56124 Pisa, PI, Italy

⁴Department of Biotechnology, School of Biotechnology and Pharmaceutical Sciences, Vignan's Foundation of Science, Technology, and Research, Vadlamudi-522213, Guntur, Andhra Pradesh, India

⁵Dr. C. D. Sagar Centre for Life Sciences, Department of Biotechnology, Dayananda Sagar College — of Engineering, Dayananda Sagar Institutions, Kumaraswamy Layout, Bangalore 560078, Karnataka, India

⁶Department of Mechanical, Chemical and Materials Engineering, University of Cagliari, Piazza d'Armi, 09123 Cagliari, CA, Italy

⁷Interdepartmental Center of Environmental Science and Engineering (CINSA), University of Cagliari, via San Giorgio 12, 09124 Cagliari, CA, Italy

***Corresponding author:** gianni.lutzu@teregroup.net

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Abstract

Nowadays one of the major environmental problems at the global level is the fact that the water is polluted by huge loads of organic and inorganic compounds, heavy metals, and chemicals produced by agricultural, industrial, and human activities. The treatment of these wastewaters (WW) represents a challenge for many countries. Among the various approaches that can be adopted to remediate WWs, an environmentally friendly and costless option is the adoption of biological technologies such as the use of microalgae-based technologies, referred to as phycoremediation. Due to the nature of WWs microalgae are able to growth and produce biomass by exploiting as nutrients some compounds that can be found in the water. In this way, they reduce the concentrations of such compounds in the WWs. The attractive fact related to the use of microalgae is that these photosynthetic microorganisms can be used in multipurpose environmental applications such as the remediation of WWs and the capture of carbon dioxide, representing at the same time a useful feedstock that can be exploited to produce food, feed, biodiesel as well as fertilizers, bio-pesticides and bio-stimulants for the agricultural sector. This review will encompass the direct utilization of microalgal biomass produced by phycoremediation as a valuable feedstock for the production of bio-stimulants, bio-fertilizers,

and bio-pesticides for the agricultural sector. An analysis of the role played by microalgae-based products as a valid alternative to traditional agrochemicals has been discussed.

Keywords: microalgae, wastewater treatment, sustainable agriculture, bio-fertilizer, bio-stimulants, bio-pesticides

1. Introduction

Chemical fertilizers and wastewater (WWs) produced by human activities are among the most urgent issues to be solved at the global level due to the increasing population, urbanization, climate change, and modern lifestyle (Fagodiya *et al.*, 2017, Lutz *et al.*, 2021). These issues push the scientific community to develop immediate sustainable solutions for preserving the natural status of the environment and the quality of human life. Huge volumes of WWs are generated daily and a large part of them is discharged into the environment without any treatment, especially in developing countries. Therefore, reduction, recycling, and reuse of untreated WW are mandatory actions to reduce the loads of pollutants in aquatic and terrestrial ecosystems. The use of algae for the removal of waste from the environment, referred to as phycoremediation, shows many advantages over other conventional remediation techniques. This environmentally friendly method of removing waste from the environment is cheap, low energy consuming, and generates a very low amount of waste (Singh and Vaishnav, 2022). In the last decades, a wide range of cyanobacteria and microalgae species including *Chlorella*, *Scenedesmus*, *Arthrospira*, *Botryococcus*, *Chlamydomonas*, *Nostoc*, *Nitzschia*, *Phormidium*, and *Oscillatoria* have been used for phycoremediation purposes (Touliabah *et al.*, 2022). One of the main advantages of using microalgae for WW treatment, besides high efficiency and safety, is that the generated biomass can be further used for several applications. These include the production of biofuels, animal feed, or bio-fertilizers and bio-stimulants for agriculture (Jayaseelan *et al.*, 2021, Srimongkol *et al.*, 2022),

Today the global market of chemical fertilizers consists of the sales of nitrogenous or phosphatic fertilizer materials, the manufacturing of fertilizers from sewage or animal waste, and the mixing of nitrogenous or phosphatic materials with other ingredients into fertilizers. The main types of chemical fertilizers, based on nitrogen (N), phosphate, and potash, can be solid, liquid, and

gaseous. N-based fertilizers are largely used in agriculture with a consequent emission in the atmosphere of greenhouse gases (GHGs) such as nitrous oxides which directly contribute to the risk of global warming and climate change. The methods of fertilizer application to crop categories such as cereals, oilseeds, vegetables, and fruits are broadcasting, drop spreading, fertigation, and foliar spray. The global chemical fertilizers market grew from \$146.7 billion in 2021 to \$158.04 billion in 2022 at a compound annual growth rate (CAGR) of 7.7%. The negative effects of long-term synthetic chemical fertilizer use on the soil and other environmental concerns boosted the use of bio-fertilizers as an environmentally friendly substitute.

A bio-fertilizer is a mix of living microorganisms that can fix atmospheric nitrogen (N) or convert soluble phosphate and potash in the soil into forms available to the plants. In this way, bio-fertilizers can promote growth in plants by increasing the supply or availability of primary nutrients to the host plant. Eukaryotic microalgae and prokaryotic cyanobacteria can capture carbon dioxide (CO₂), fix N, and improve soil fertility. Owing to these abilities, algal fertilizer can be considered as a category of organic fertilizer. Many studies explored the effects of algal bio-fertilizer on the microbial community in soil ecosystems and the contributions of algal bio-fertilizer to N fixation, providing a deep understanding of the positive roles of algal bio-fertilizer in eco-friendly agriculture (Kang *et al.*, 2021). Furthermore, other healthier aspects linked to the use of organic fertilizers of animal and vegetable origin are summarized in Figure 1 as follows: a) the improvement of soil structure, root development, and rise in soil pore water as a consequence of the increase in nutrient interaction and soil microbial activities (driven by the increase in nutrient interaction and soil microbial activities); b) the improvement of the soil-biological activity, which favors the mobilization of nutrients from organic substances to soil; c) the increase in the organic content of soil and hence in the nutrient exchange capacity, N-fixation, P solubilization, soil-water retention, soil aggregation, and buffering capacity against acidity and alkalinity; and finally d) the promotion of microorganisms and earthworms growth in the soil (Kumar *et al.*, 2021). Among the most common microalgae-derived products currently available in the market there are bio-stimulants. These products are in high demand, especially in the organic farming sector. Their potential use to obtain increased yields or improved fruit quality has been demonstrated in several recent reports (Colla, 2020; Gonz  les-P  rez *et al.*, 2022). According to a report available on GlobalNewswire Inc, the market of bio-fertilizers generated worldwide revenue of \$ 2,391.51 million in 2021, and is expected to generate \$

4448.97 million by 2028, witnessing a CAGR of 11% from 2022 to 2028 (GlobalNewswire, 2023). To date, most of the prominent players in the bio-fertilizers market are located in Asia (13), followed by Europa (6) and North America (4), as reported in **Table 1**. North America is forecasted to be the fastest-growing market at the global level. This trend is mainly due to the growth in the farmers' demand for bio-fertilizers and advanced irrigation systems like drip and sprinkler irrigation for fertigation. Moreover, growing concerns affecting the excessive use of chemical fertilizers and nitrate emissions are some of the prime factors expected to contribute to the growth of bio-fertilizers not only in the USA but also in Mexico and Canada.

Insert here Table 1

Owing to the ability of algae to recover nutrients from WW, a novel concept of phycoremediation and exploitation of biomass as bio-fertilizers is recently gaining more attention as an emerging technology for eco-friendly agriculture. This new concept leads to a microalgal biorefinery approach, which is the prime requirement for the sustainable production of bio-fertilizers after the remediation of WWs. The benefits potentially assured by the microalgal biorefinery approach are not only related to human and environmental health in terms of GHGs emissions and phycoremediation but entail also positive implications in the fields of renewable energy, food security and mitigation of climate changes (Osorio-Reyes *et al.*, 2023). The availability of WW at a very low cost as feedstock for producing algal bio-fertilizers which can be sold at reduced prices, the possibility to convert waste nutrients to value-added biomass creating economic benefits, a dramatic potential reduction of WW pollution by algae nutrients recovery, and the exploitability of harvested biomass as algal bio-fertilizer for eco-friendly agriculture are some of the great advantages linked to WW remediation for bio-fertilizers production (Kour *et al.*, 2020). In the literature several recent examples of phycoremediation + bio-fertilizers obtained from algae produced as a result of WW phycoremediation are available. Khan *et al.* (2019) proposed the growth of three algal species (*Chlorella minutissima*, *Scenedesmus* sp., and *Nostoc muscorum*) in a sewage WW for nutrients removal and production of bio-fertilizers as harvested value-added biomass. Sharma *et al.* (2021) coupled the treatment of sewage WW by *C. minutissima* with the addition of derived algal biomass to the soil for the cultivation of baby corn and spinach. Alvarez-González *et al.* (2022) successfully improved the production of basil crops using as fertilizer algal biomass obtained by *Scenedesmus* sp. grown in

a domestic WW. Ferreira *et al.* (2021) carried out a comparative investigation to evaluate the ability of three microalgae including *Tetradescmus obliquus*, *Chlorella protothecoides*, and *C. vulgaris*, and the cyanobacterium *Synechocystis* sp. to treat piggery WW. The obtained microalgal biomass was tested to evaluate its effect on germination, root and shoot growth, and pigment content in seeds of watercress, tomato, cucumber, barley, wheat, and soybean. Overall, the roles of microalgae-based bio-fertilizer in the sustainability of green agriculture and food-water-energy- security nexus have recently been reviewed by Cao and co-workers (2023).

2. Algae-based wastewater remediation

The major sources of water pollution are represented by human settlements, industry and agriculture. In the frame of a circular bio-economy context the use of microalgae for the treatment of WWs, mainly those related to agriculture, is considered a winning option by taking into account not only the capability of these photosynthetic microorganisms to remove pollutants from the water but also in the light of the multipurpose uses that can be reserved to the obtained algal biomass (production of bio-fertilizers, biofuels, biochar, food, and feed bio-products). Microalgae removal efficiencies rates in terms of total nitrogen (TN), total phosphorous (TP), and chemical oxygen demand (COD), as well as biomass production yields for the most recent reports available in the literature, are summarized in **Table 2**. Most of the data rely on *Chlorella* and *Scenedesmus* strains since these last are considered as robust microalgae able to adapt to a wide range of WWs streams.

Insert here Table 2

2.1 Wastewater from agricultural run-off

The demand for food production is constantly increasing due to the rapid growth of population. In parallel, there is a worldwide need to increase crop yields to feed the 8 billion inhabitants of the Earth (Abuhai *et al.*, 2023). The enormous pressure on agricultural lands can be supported by using biofertilizers and biopesticides along with other management practices. The use of soil improvers makes agriculture a key player in water pollution, accounting alone for 70% of water abstractions worldwide. Water bodies around farmlands receive daily not only agrochemicals but also large quantities of organic matter, increased concentrations of N, P, and other mineral inputs, drug residues, sediments, and saline drainage, which contribute to severe environmental

degradation of ecosystems and represent a serious risk for human health and productive activities (UNEP, 2016). To date agriculture is responsible for the contamination of water bodies in Europe (38%), and in the USA which represents the main source of pollution in rivers and streams. Also, the pollution in wetlands and lakes is responsible for rendering the groundwater to be unfit for human consumption (Mateo-Sagasta *et al.*, 2017).

In order to mitigate nutrient load in water bodies, removal of the excess of N species and pesticides at the source, can be adopted using, riparian buffers, saturated buffers, denitrifying bioreactors, and twin-layer cultivation systems (Coppolino *et al.*, 2022; Sohail *et al.*, 2023).

Among the phytoremediation technologies for pesticide and nutrients attenuation in agricultural run-offs (construction of buffer strip, wetlands or algae-based ponds), the cultivation of microalgae in wetlands, open ponds and bioreactors is receiving today a great attention (Sohail *et al.*, 2023). One of the reasons for the growing popularity of this technology is that microalgae treatment of agricultural-run-offs allows the cleaning of the water while generating at the same time biomass that can be used as bio-fertilizer (Wei *et al.*, 2023).

While several lab-scale experiments have been carried out using microalgae cultures for agricultural run-off treatment, only recently some studies have evaluated the feasibility of integrating this treatment with biomass production by microalgae at large scale (Chauhan and Prajapati, 2022).

In a very recent work by Sohail *et al.* (2023) the phycoremediation potential of *Tetradesmus obliquus* for the treatment of several kinds of high-nutrient WWs filtered and diluted by wetland WW was highlighted. The strain cultured in poultry WW achieved the maximum removal efficiency for NH_4^+ , Total-N and P-PO_4^{3-} equal to 6.15, 7.64 and 0.6 $\text{mg L}^{-1} \text{d}^{-1}$ respectively. This wetland WW dilution system also provided the highest productivity, C capture, and the highest lipid content of 5.13, 9.25, and 1.48 $\text{g m}^{-2} \text{d}^{-1}$ respectively. Comparing the phytoremediation efficiency of the strains *Chlorella minutissima*, *Scendesmus* sp., *Nostoc muscorum* and their consortium, other authors reported that the first strain was the most efficient. Indeed *C. minutissima* reached the highest removal efficiency for $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, BOD_5 and COD were 87, 92, 85, 90 and 81% respectively. *C. minutissima* showed the greatest biomass production followed by *Scendesmus* sp. and *N. muscorum*, with 0.45, 0.44, 0.14 g L^{-1} respectively (Khan *et al.*, 2019). The association of bacillus and microalgae bio-fertilizers also improves the quality and quantity of *Salvia miltiorrhiza* production (Wei *et al.*, 2023). The

integrated spontaneous microalgae and constructed wetland system shows NH_3 , TN, soluble P removal efficiency of 99.2%, 71%, and 61% (Celente *et al.*, 2019).

2.2 Hydroponic effluent wastewater

The total area in use for hydroponic systems increased significantly, from 23 ha in 1993 to 1107 ha in 2008, and by now it has probably at least doubled (Lee *et al.*, 2018). The recycled hydroponic water needs to be discharged due to increased salinity and remaining ions that have not been assimilated by crops (Hultberg *et al.*, 2013). These hydroponic WWs are rich in mineral compounds, such as nitrate, phosphate and sulfate, and are characterized by relatively high concentrations of major ions (e.g., K^+ , Mg^{2+} , Ca^{2+}) compared to municipal and industrial WW. They also showed low amounts of organic matter (Delrue *et al.*, 2021; Lee *et al.*, 2018).

There are several alternative strategies to conventional hydroponic effluents WW treatments for the sustainable management of effluents from open- and semi-closed soilless growing systems. A successful method may be the phytoremediation using constructed wetlands (Prystay and Lo, 2001). Microalgae-based WW treatment is considered the most promising technology for the treatment and nutrient recovery of WW (Richa *et al.*, 2020). Photosynthetic microorganisms including microalgae have been extensively investigated for N and/or P removal in industrial, municipal, and conventional agricultural WWs (You *et al.*, 2022). However, there have been relatively few investigations regarding the use of microalgae in hydroponic WW treatment and they are scarcely comparable to each other for the diversity of experimental designs. The most used microalgae species is certainly *Chlorella vulgaris* or *Chlorella* sp., which in some cases was studied in comparison with *Scenedesmus* sp. (Baglieri *et al.*, 2016; Delrue *et al.*, (2021) or *Parachlorella* sp. (Lee *et al.*, 2018). Saxena and Bassi (2012) evaluated the marine microalgae *Dunaliella salina*. In most cases, the experiments were conducted in laboratory under controlled conditions or at room temperature (25-30 °C) (Baglieri *et al.*, 2016; Bertoldi *et al.*, 2008; Delrue *et al.*, 2021; Saxena and Bassi, 2012; Yousif *et al.*, 2022; Vazirzadeh *et al.*, 2022). Lee *et al.*, (2018) conducted their experiment both in laboratory and in plant factory, while other authors conducted the experiments in a greenhouse (Cowan *et al.*, 2022; Hultberg *et al.*, 2013; Huo *et al.*, 2020). In all the experiments, the effluent volume used was relatively small ranging from 10 ml (in microplates) to 0.4-2.0 L ml in flask or 1.5 – 10 L in bottles (Cowan *et al.*, 2022; Huo *et al.*, 2020).

The characteristics of the tested effluents in the aforementioned studies were quite different. For instance, Vazirzadeh *et al.*, (2022) used an optimal growth medium for algae, the sterilized BG11, as a simulated agricultural runoff; instead, an artificial, non-sterile and nitrate-rich WW or a commercial nutrient solution were used by other authors (Huo *et al.*, 2020; Lee *et al.*, 2018). In some studies, the tested WW was an artificial nutrient solution with ion composition almost identical to the genuine effluent (Hultberg *et al.*, 2013; Vazirzadeh *et al.*, 2022) or sterilized (Saxena and Bassi, 2012), filtered (Hultberg *et al.*, 2013) or diluted effluent (with distilled water by 50 to 75%) (Bertoldi *et al.*, 2008). Many authors compared diluted and undiluted crude hydroponic effluents, after sterilization, in laboratory experiments (Bertoldi *et al.*, 2008; Delrue *et al.*, 2021). Most of the experiments were conducted under controlled conditions in laboratory, except for those carried out by Hultberg *et al.* (2013), Cowan *et al.* (2022) and Huo *et al.* (2020). The removal efficiency of N and P ranged from 21 to 100% and from 70% to 100%, respectively. The biomass produced in the different works cannot be compared because it is expressed with different measuring units, such as cell number (Saxena and Bassi, 2012; Bertoldi *et al.*, 2008), optical density (Lee *et al.*, 2018) and dry mass per volume of growing media (g L^{-1}). Values of 3.75 and 3.65 g L^{-1} were obtained in cultures of *Chlorella vulgaris* and *Scenedesmus quadricauda*, respectively (Baglieri *et al.*, 2016). Other authors reported values ranging from 0.3 to 3.0 g L^{-1} (Delrue *et al.*, 2021; Yousif *et al.*, 2022; Hultberg *et al.*, 2013; Huo *et al.*, 2020). A work that stood out from all was that of Salazar *et al.* (2021), which started with a screening of 13 Nordic microalgae grown on a filtered, low conductivity hydroponics effluent. Then, the 13 strains were cultivated under controlled conditions in small volumes (2 ml) to choose a single strain. Subsequently, this strain was checked for nutrient removal efficiency and microalgal biomass production in 500 ml flasks in laboratory, and finally in a single 65 L automated photobioreactor system with CO_2 flow, kept in greenhouse.

2.3 Dairy wastewater

In the last decade, the dairy industry has undergone different changes, whose technological innovations have made it possible to produce dairy foods with health

benefits, increase shelf life, improve the transport and storage efficiency of milk, and develop new marketing and packaging strategies.

According to the Food and Agriculture Organization (FAO), there has been an increase in the consumption of dairy products in recent years, and they are expected to increase by 13.7% by 2023 (FAO, 2016a). However, the dairy industry uses great amounts of water for all production lines. It is appraised that the amount of WW produced by the dairy industry is about three times the quantity of processed milk with about 0.2-10 L of DWW produced for every 1 L of milk (Ye *et al.* 2023).

DWW contains a complex mixture of different inorganic nutrients at high concentrations (phosphates, nitrogen-ammonia), oils and fats, BOD, COD, and both dissolved and suspended solids (Sudhanthiran and Perumalsamy, 2022, Sathinathan *et al.*, 2023). However, since these dissolved substances represent a danger both to public health and to the environment, such wastes must necessarily be treated before disposal (Ganta *et al.*, 2022). The most important issues in the treatment of DWW are the reduction of oxygen demand, which can reach between 2000-2500 mgL⁻¹ of COD and 800 mg L⁻¹ of BOD, and the reduction of contaminants with potentially harmful effects on the health (i.e., antimicrobials, antibiotics, hormones used in livestock production) (Kang *et al.*, 2022). Conversely, organic materials, including fats, lactose, and proteins (such as casein) are harmful to the environment (Ganta *et al.*, 2022). These substances are mainly derived from bottling and packaging processes, together with cheese and ice cream production cycles.

Generally, DWW is treated through physical, chemical, or biological methods depending on the composition of the effluent (Sathinathan *et al.*, 2023). Due to the high energy requirement and the large amount of chemicals to be used, these methods of DWW treatment are not economical (Sathinathan *et al.*, 2023, Das *et al.*, 2023). One of the disadvantages of physic-chemical methods can be secondary contamination which can be caused by the safe disposal of contaminated by-products.

Anaerobic digestion processes are common treatments for DWW but their effluent needs additional nutrient removal steps as it may cause eutrophication of watersheds (Li *et al.*, 2019). As a rule of thumb, the treatment of DWW has been done by adopting conventional techniques such as setting ponds and oxidation. These techniques have different problems, but the main one is represented by the high energy consumption with a low nutrient removal efficiency. For this

reason, new technologies based on microalgae are being developed for the treatment of WW (Sudhanthiran and Perumalsamy, 2022, Glover *et al.*, 2023).

Microalgae due to rapid cell growth rate and the capability to assimilate both inorganic and organic compounds -as well as a high quantity of nutrients, have been studied for DWWs treatment. At the same time, they can generate many benefits, such as lower CO₂ emissions, low production costs, lower energy consumption, and use of chemical compounds. The resulting biomass can be used in energy and bioplastics industries (Chong *et al.*, 2021, Lutz *et al.*, 2021). In recent work, Kanagam and Rajasekaran (2023) investigated the use of three species of microalgae, *Desmococcus olivaceus*, *Scenedesmus dimorphus*, *Chlorella vulgaris* for DWW treatment. In the study of Khalaji *et al.* (2023) *C. vulgaris*, grown at different starting inoculums and DWW concentration, was evaluated for nutrient removal efficiency and biomass and lipid production. Iliopoulou *et al.* (2022) instead analyzed the use of different types of DWW for the cultivation of *C. sorokiniana* and assessed the production of biomass and biomolecules as well as the reduction of nutrients and COD. COD and ammonium were reduced by 91% and 80% respectively. *C. sorokiniana* was investigated in a recent publication by Kusmayadi *et al.* (2022) for the optimization of the phycoremediation process.

2.4 Livestock and aquaculture wastewater

According to reports sponsored by the Food and Agriculture Organization of the United Nations (FAO) the areas devoted to irrigation has more than doubled in the last fifty years rising from 139 Mha (million hectares) in 1961 to 320 Mha in 2012 (FAO, 2014), the cattle has more than tripled passing from 7.3 billion units in 1970 to 24.2 billion units in 2011 (FAO, 2016a), while only in the last forty years the aquaculture industry has grown more than 20-fold, particularly in Asia (FAO, 2016b). Today meat represents an important source of protein for the daily human's food requirements and in particular pork and chicken consumption has been increasing continuously worldwide. Limiting the discussion to pigs, starting from 2010 about one billion of hogs were raised every year for food markets (Ha *et al.*, 2023). The thousands of swine farms distributed on the five continents make problematic and unsustainable their management due to the occupation of large land areas, the consumption of energy and a strong adverse environmental impact linked to the release of greenhouse gases (GHG) in the atmosphere potentially risking human health (Hollas *et al.*, 2023). Swine wastewater (SWW) derives mainly

from the cleaning of manure, closed and open animal housing which if untreated can cause water eutrophication and pollution, soil pollution, odour related problems, harbour resistant genes/bacteria (Zhao *et al.*, 2023; Ibekwe *et al.*, 2023). SWW is a waste particularly rich in P (50-230 mg L⁻¹) and N (800-2300 mg L⁻¹) with an N:P ratio of 15 and some other metal ions (Nia *et al.*, 2023; Kannan *et al.*, 2023). In addition, SWW can contains substances like high quantity of suspended solid, organic material and toxicants such as heavy metals, antibiotics and hormones. To date the treatment methods for decreasing total nitrogen (TN), total phosphorus (TP), BOD, and COD levels of SWW proposed by regulatory agencies, such as the American Environmental Protection Agency (EPA), are not feasible due to the high costs of energy and the management that must be assured for handle the secondary waste streams. Nowadays, anaerobic digestion represents the most used process for the treatment of solid waste found in a SWW as it allows the conversion of organic matter into biogas (Pu *et al.*, 2022). Recently, microalgae cultivations in WW for the removal of contaminants and the production of microalgal biomass have been identified as new treatment technologies that help for global climate change mitigation (Liu *et al.*, 2022a; Wang *et al.*, 2022b).

A recent study established that the cultivation of microalgae in a raw SWW is not viable and that the substrate must undergo different pre-treatment steps to attain valid results. This is the case described by Osabutey *et al.* (2023) where microalgal growth and N and P removal efficiency in *Scenedesmus dimorphus* were higher in the pre-treated WW in contrast to the raw SWW. Similarly, this effect was described in the study of Muthuraman *et al.* (2023) in which the growth and productivity of *Coelastrella* sp. were hindered in a not pre-treated substrate. In another research the interference of zinc with algal growth was shown (Oliveira *et al.*, 2023). They described how high concentrations of zinc led to a reduction in the removal of ammonium, while P was knocked down. Regarding to the production of lipids, the study by Das *et al.* (2022) stated that growing microalgae, *Desmodesmus maximus*, in SWW led to an increase in the content of lipids, which can be used for biodiesel production. An innovative study proposes the use of a consortium of microalgae and bacteria for the treatment of SWW (Chen *et al.*, 2022). Initially the WW was inoculated with bacteria to carry out an initial treatment, then microalgae were added to complete the task. The results showed a reduction of COD, BOD, TN and TP of 72.1%, 94.9%, 88% and 94.6%, respectively. At the same time, the resulting biomass contained 36% of carbohydrates, an interesting result to produce biochar. Another consortium was studied for its

ability to reduce the concentration of pollutants such as oxytetracycline (OTC), ofloxacin (OFLX) within the SWW (Zhang *et al.*, 2022b). The symbiosis between *Dictyosphaerium* and *Pseudomonas* allowed a biomass production of 3.5 g L⁻¹ and a percentage of removal of COD, TAN, TP, OTC, OFLX of 83.4%, 62.4%, 100%, 100%, 60.1% respectively. Some researchers compared *Chlorella* sp. with different strains of nitrifying heterotrophic bacteria, to evaluate which strain was better at removing nutrients from a SWW. Microalgae proved more efficiency than bacteria at removing ammonium and TN (80.9% and 66%), while nitrifiers were more effective at removing COD and P (83.9% and 76.2%) (Liu *et al.*, 2022b).

3. Microalgae-based products for a sustainable agriculture

3.1 Application of algae in soil as bio-fertilizers

Bio-fertilizers consist of mixtures of live or dormant single or multiple strains of microorganisms (algae, bacteria, and fungi) that enhance the plant growth and the nutrient availability at the seeds, plant surface, or soil level. They differ from chemical fertilizers which consist of mixtures of various elements and minerals needed to enhance plant growth and produced from synthetic materials, with organic fertilizer coming from the natural decomposition of organic wastes produced by plant or animal sources (such as green manure). Bio-fertilizers can colonize the rhizosphere and the interior of the plant, fixing atmospheric N, decomposing organic material, and increasing the solubility of soil minerals. They are not only responsible for enhancing soil physiochemical properties but also for affecting the structure, function, and abundance of soil native microorganisms through changes in microbial diversity and communities (Aponte *et al.*, 2022). It has been reported that algal extracts contain high essential micro and macronutrients (Zn, K, Mg, Ca, P, S, K, Zn, Mo, Cu, Co, and Fe), several regulators and growth-promoting hormones, enhancers, polyamines and vitamins able to favor a healthy development of crops, by increasing nutritional status, vegetative development, yield, and fruit quality in various orchards and vineyards. Several phytohormones, phenolic compounds, polysaccharides, terpenoids, free fatty acids, and alpha and carotenoids are produced by microalgae and cyanobacteria (Ravishankar and Ranga Rao *et al.*, 2019a; 2019b; Sharma *et al.* 2010). Therefore, algal biomass can serve as a source of nutrition as well as a carrier material to form bio-fertilizers (Allouzi *et al.*, 2022).

Insert here Table 3

In **Table 3** a list of the most recent studies on the use of algal biomass as soil bio-fertilizers is reported, with particular emphasis on the improved chemical and nutritional properties of the amended soils, fruits quality and stress tolerance, and producing growth promoting substances. Some algal metabolites have been found to stimulate plant growth directly or indirectly by interacting with soil microbes for bio-mineralization or plant-microbe symbiosis, thereby increasing nutrient availability. This aspect has been reported for lettuce seedlings grown by applying cellular extracts of *C. vulgaris* and *Scenedesmus quadricauda* (Puglisi *et al.*, 2020b). Gougoulas *et al.* (2018) investigated the effect of dried *C. vulgaris* biomass mixed with sheep manure which were added to the soil and incubated for 15 weeks to detect the role of adding small amounts of algal biomass to the soil in biodegradation of soil organic C. The mineralization of soil organic C increased by 16.2–35.9% at all rates of algae addition compared to the control. There was a 40–50% rise in the available form of K, while the corresponding content of nitrate N increased by 20–30%. The available forms of Cu, Mn, and Zn were also increased by 56.8–61.9%, 55.8–67.3%, and 34.1–40.6%, respectively. The results proved that *C. vulgaris* biomass can act as an accelerator agent in the biodegradation of soil organic matter, without any significant negative impact on soil chemical properties. Extracts of *C. vulgaris* and *Scenedesmus quadricauda* were also applied directly into the soil to monitor their effect on the complex soil-microorganism-plant system. Barone *et al.* (2019) carried out experiments in pots using agricultural soil and tomato plants.. The effects of the addition of *C. minutissima* biomass from phycoremediation to the soil in field plots of baby corn and spinach on soil chemical properties as well as plant growth and yield was investigated also by Sharma *et al.* (2021). The application of 100% N fertilizer by algal biomass led to a higher economic yield of spinach and baby corn when compared to the recommended dose of synthetic fertilizers. The available N and P content in experimental plots applied with algae biomass as bio-fertilizers was significantly higher than in other treatments. The soil supplied with 100% N by algae biomass significantly increased the activity of some enzymes (urease, nitrate reductase, and dehydrogenase) in spinach and bay corn grown soil. The effect of algae bio-fertilizers on crops growth and soil fertility was analyzed both at field and greenhouse level. Alobwede *et al.* (2019) tested the effects of green algae *Chlorella* sp. and cyanobacterium *Arthrospira platensis* (*Spirulina*) addition to arable soil in greenhouse-grown garden peas and field plots of spring wheat on plant growth and nutritional,

physical and chemical properties of the soil. In the greenhouse at the highest application rates (4 g kg^{-1}), *Chlorella* sp., and *Spirulina* increased soil total N and available P, and *Spirulina* also increased soil nitrate concentrations. *Chlorella* sp. significantly increased soil total P, N and C, available P, $\text{NH}_4^+\text{-N}$, and pea yield. On the other hand, soil water-stable aggregates were kept unchanged by the algal additions in both the greenhouse and field study.

3.2 Application of algae as crop bio-stimulants

The bio-stimulant action on plants is carried out through a series of molecules and organisms that can be broadly divided into five main groups characterized by a very diverse nature and physiological functions. They include: a) humic and fulvic acids, b) protein hydrolysates and other N-containing compounds, c) chitosan and other biopolymers, d) inorganic compounds, e) beneficial fungi and bacteria. For most of them, examples of enhancement in cellular, molecular, physiological, and environmental aspects are provided.

Humic acids (HA) can promote cellular mechanisms (i.e. interaction with cellular components and processes) such as cell wall loosening and cell elongation in roots by activating plasma membrane proton pumping ATPases. Moreover, HA can boost physiological functions (i.e. action on whole plant processes) such as increasing linear growth of roots and root biomass. Even agricultural functions (i.e. output traits relevant for crop performance) can be promoted by HA through the increase of root foraging capacity and enhancing nutrient use efficiency. Finally, economic and environmental benefits can be obtained by using HA (i.e. changes in yield, quality, ecosystem services) through higher crop yields, saving of fertilizers and reducing losses to the environment (Jindo *et al.*, 2020).

Protein hydrolysates (PH) act on cellular mechanism stimulating phenylalanine ammonia-lyase enzyme and gene expression and enabling the production of flavonoids under stress on physiological functions with the protection against UV and oxidative damage, show their agricultural functions by increased crop tolerance to abiotic stress (i.e. salt), and their economic and environmental benefits assuring higher crop yields. Biopolymers can act on cellular mechanism by protecting photosystem II against photo damage via activation of scavengers of reactive oxygen and, at physiological level, by maintaining leaf photosynthetic activity under salt stress. In general an increase of crop tolerance to abiotic stress and favoring higher crop yields is

observed as a result of the use of PH. The beneficial action of plant promoting fungi/bacteria manifests through the release of auxins and the activation of auxin-signaling pathways involved in root morphogenesis, increasing lateral root density and surface of root hairs, increasing root foraging capacity and enhancing nutrient use efficiency, and at environmental level assuring higher crop yield, reducing the use of chemical fertilizers and therefore their dispersion into the environment (Colla *et al.*, 2017)

Algal and botanical extracts can also act as bio-stimulants in different ways. They can modulate the expression of genes encoding transporters of micronutrients, increase tissue concentrations and root to shoot transport of micronutrients, improve mineral composition and enhance nutritional value of plant tissues. The beneficial influence of microalgal extracts on plants growth has been linked to the positive effect on gibberellin, auxin, and cytokinin plant hormones (Navarro-López *et al.*, 2020). Microalgae themselves produce gibberellins, auxins, cytokinins and other hormones which have been identified in several species such as *Chlorella*, *Scenedemus*, and *Acutodesmus* genus (Stirk *et al.*, 2013; Lin *et al.*, 2022; Wang *et al.*, 2022a). Gibberellins (divided into 7 forms GA₁₋₇ depending on the position of doubled bond) can act at root, stem, flower, fruit levels by promoting plants growth. Once gibberellins production is shut down (as in mutants), the elongation of roots, stems, and floral organs is reduced (Castro-Camba *et al.*, 2022). Gibberellins have been reported to inhibit salt-induced growth in rice plants. Rodriguez and coworkers (2006) found out that the extracellular products of the cyanobacterium *Scytonema hofmanni* produced gibberellin-like plant growth regulators, which were able to enable the homeostasis of rice seedlings under salt stress. In microalgae, gibberellins can participate in cell elongation and affect the growth and cell metabolism by regulating carbon metabolic pathways (Wang *et al.*, 2022a). Auxins (such as indole-3-acetic acid, indole-3-butyric acid, phenylacetic acid) promote growth responses in plants and are involved in most of the quantitative changes taking place during the plant life cycle (Zhang *et al.*, 2022a). Their biological functions in microalgae are similar to those in higher plants. Cytokinins (*trans*zeatin, kinetin, *N-N'*-diphenylurea) regulate plant growth, physiological activities, and yield and are involved in plant response to abiotic stress (Fathy *et al.*, 2022). In microalgae they promote cell division, enhance photosynthetic pigment accumulation, and improve photosynthetic efficiency leading to an accumulation of biomass (Wang *et al.*, 2022a).

Insert here Table 4

In **Table 4** the most recent applications of microalgae as bio-stimulants are summarized. Microalgae are considered in terms of dried biomass or living cells in solution as soil improvers and foliar plant stimulants. Seed germination, content of chlorophyll, carotenoids and proteins, nutrient uptake, root and shoot length and dry weight, flowering, nutritional content of fruits, plant nutrition status, and influence on the activity of plant enzymes are some of the effects considered on crops development. Umamhesvari and Shanthakumar (2020) investigated the use of live algal biomass from *C. pyrenoidosa* grown in paddy-soaked rice mill WW as bio-fertilizer for the primary seedling experiments of Indian okra (*Abelmoschus angulosus*). These experiments involved soil amendment treatment samples T1 (control soil – no amendment), T2 (soil + live algal biomass), and T3 (soil + chemical fertilizer). It was found out a maximum absolute growth rate of 0.40 day^{-1} in T2 plants with better growth characteristics of chlorophyll, fresh and dry shoot, root weights (which were ~30% more than that of T3), and control soil (T1) does not exhibit the plant growth as the soil was low in nutrients. A similar investigation was carried out using the biomass of the filamentous cyanobacterium *Arthrospira platensis* (*Spirulina*) cultivated in aquaculture WW (Wuang *et al.*, 2016). This biomass was applied as bio-fertilizer on Arugula (*Eruca sativa*), Bayam Red (*Amaranthus gangeticus*), Pak Choy (*Brassica rapa ssp. chinensis*), and Kai Lan (*Brassica oleracea alboglabra*) plants. The results showed an increase in plant height by 55.3% and chlorophyll content by 30.2% by Arugula plants when compared to plants grown in traditional chemical fertilizer. *Spirulina* biomass had also a beneficial effect on root length in Pak Choy plants and in terms of seedlings dry weight in Kai Lan plants.

3.3 Microalgae-based bio-pesticides

The use of bio-pesticides obtained by microalgae can expand crops tolerance not only toward abiotic stresses such as high salinity, drought, and frost but mainly toward biotic stresses represented by pathogens, pests, and insects (Asimakis *et al.*, 2022). Plant pathogens represent a serious threat for crops stability and jeopardize the market of agricultural products with thousands of dollars in losses annually due to the destruction of entire crops. Pathogens such as bacteria, viruses, fungi, nematodes, and insects can cause many diseases in plants at foliar, stem, root, and vascular level. The feasibility of using the antibacterial and antifungal activity of

microalgae and cyanobacteria is well established (Parsaei-mehr and Lutz, 2016; Berthon *et al.*, 2021; Horváth *et al.*, 2023). In addition, microalgae and their extracts also display nematocidal (El-Deen and Issa, 2016), herbicidal (Chaib *et al.*, 2021), insecticidal (Saber *et al.*, 2018) and acaricidal (Ruangsomboon and Pumnuan, 2016) properties against a wide range of crop pathogens.

As far as algae are concerned, many studies demonstrated how some seaweed strains can efficiently suppress the biological activity of plant pathogenic bacteria belonging to the genera *Agrobacterium*, *Pseudomonas*, *Xanthomonas*, and *Erwinia*, that are responsible for most of the serious diseases that affect important crops such as rice and potato (Righini *et al.*, 2019). A comprehensive list of the top 10 bacterial plant pathogens was provided in 2012 by Mansfield and co-workers. It included in rank order: 1) *Pseudomonas syringae*, 2) *Ralstonia solanacearum*, 3) *Agrobacterium tumefaciens*, 4) *Xanthomonas oryzae* pv. *Oryzae*, 5) *Xanthomonas campestris*, 6) *Xanthomonas axonopodis*, 7) *Erwinia amylovora*, 8) *Xylella fastidiosa*, 9) *Dickeya dadantii* and *solani*, 10) *Pectobacterium carotovorum* and *Pectobacterium atrosepticum*.

Recently Shaima *et al.* (2022) investigated the chemical nature of some algal bioactive compounds in terms of antimicrobial properties against both Gram-positive and Gram-negative bacteria. Methanol extracts were obtained from *Chlorella sorokiniana* (UKM2), *Chlorella* sp. UKM8, and *Scenedesmus* sp. UKM9. *Chlorella* sp. demonstrated the highest antibacterial activity.

Algae display the same inhibitory action against the growth of myceliums by conferring resistance in plants against the most widespread fungal genera (*Fusarium*, *Verticillium*, *Rhizoctonia*, *Phytophthora*, and *Phoma*) (Esserti *et al.*, 2017). The fungicidal properties of aqueous extracts from four microalgae strains (*Nannochloropsis* sp., *Phaeodactylum tricornutum*, *Scenedesmus obliquus*, *Chlorella vulgaris*) and one cyanobacterium (*Spirulina* sp.) were investigated, *in vitro*, by Schmid *et al.* (2022) against the most problematic phytopathogenic fungi for modern agriculture, namely *Sclerotium rolfsii*, *Rhizoctonia solani*, *Botrytis cinerea* and *Alternaria alternata*. The highest antifungal activity against *S. rolfsii* was exhibited by *S. obliquus* ($32.01 \pm 4.82\%$) followed by *P. tricornutum* ($18.35 \pm 3.45\%$) and *Nannochloropsis* sp. ($13.96 \pm 5.26\%$). Moreover, *P. tricornutum* suppressed also the mycelial growth of *R. solani* ($18.35 \pm 3.45\%$) and *B. cinerea* growth ($11.47 \pm 2.06\%$).

Tobacco mosaic virus (TMV) and potato virus X (PVX) are two common viral pathogens which can be successfully inhibited by the antimicrobial activity of algae, either directly or by inducing plant defense mechanisms, as demonstrated by Jimenez *et al.* (2011), who performed an analysis of the effects of aqueous and ethanolic crude extracts from nine different seaweeds. Besides the antimicrobial activity of macroalgae against plant pathogenic viruses, to the best of our knowledge examples of biological control of pathogen viruses mediated by microalgae are not available until now in literature. Therefore, it is important to fill this gap by testing the potential application of microalgae against the most widespread plant viruses.

Microalgae-based bio-pesticides are efficiently tailored not only against pathogenic microorganisms but also having animals as target. In fact, algal extracts have been tested against soil-borne nematodes, plants or fruit feeding insects, larvae, and mites. Plant-parasitic nematodes are considered a major pest in grape growing countries. *C. vulgaris* extracts applied through soil-drench to grapevine roots reduced by 2-3 fold the infestation by the root ectoparasite nematode *Xiphinema index* (Bileva, 2013). The use of microalgae *Scenedesmus obliquus* and *Chlorella vulgaris* as well as cyanobacterium *Anabaena oryzae* has been reported against the growth of nematode *Meloidogyne incognita* in banana plants (Hamouda and El-Ansary, 2017). All the three strains were able to reduce the development of the second stage juveniles in the soil.

Ruangsomboon and Pumnuan (2016) carried out an investigation to evaluate the anti-acaricidal activity of red, green, brown macroalgae and seven crude cyanobacteria extracts (*Fischerella* sp., *Hapalosiphon* sp., *Mastigocladopsis* sp., *Oscillatoria* sp., *Phormidium* sp., *Spirulina maxima* and *S. platensis*) against the house dust mite *Dermatophagoides pteronyssinus* (Trouessart). The crude extracts were isolated from cyanobacteria by means of serial extractions with methanol, dichloromethane, and hexane and were tested through the contact method at three concentrations (0.32, 3.2, and 32 $\mu\text{g cm}^{-2}$) using 95% ethanol as a control. It was found out a 72.5-99.3% mite mortality with methanolic crude extracts of *Oscillatoria* sp., *Phormidium* sp., *Spirulina platensis*, and *S. maxima* at a concentration of 32 $\mu\text{g cm}^{-2}$. Based on these results, the range of concentration for these extracts was re-arranged at 0, 0.16, 0.32, 1.6, 3.2, 16, 32, 48, and 64 $\mu\text{g cm}^{-2}$. The highest acaricidal activity was showed by *Oscillatoria* sp. and *Phormidium* sp., with LC_{50} values of 0.39 and 0.40 $\mu\text{g cm}^{-2}$, respectively, suggesting that these two cyanobacteria might be successfully used to contrast this house infesting mite. The feasibility of using microalgae extract against insects infesting crops was early reported by Sathiyamoorthy and

Shanmugasundaram (1996) which applied glycine-rich peptides (0.01-0.1%) extracted from the cyanobacterium *Scytonema* MKU106 to the leaves of cotton plants in order to reduce the feeding frequency of the leaf-roller chewing moth *Sylepta derogata*. Cotton plants can also be infested by the leafworm *Spodoptera littoralis* mainly at larval and pupal stage. To contrast this moth, crude ethanolic extracts of heterocytous cyanobacterium *Nostoc carneum* and of green algae *Parachlorella kessleri* were tested by using the contact method in vitro (Saber *et al.*, 2018). All the treatments showed insecticidal activity against the larvae corroborating the idea to use microalgae extracts as ecofriendly bio-pesticides for pest management of cotton crops.

Insert here Table 5

Table 5 provides examples about the use of natural products against pests (bacteria, virus, insects, nematodes) in agricultural practices. All the chemicals extracted from algal biomass (peptides, fatty acids, terpenes, essential oils) exhibit significant advantages compared to the chemical ones. First of all these natural chemicals are highly biodegradable and safe from an environmental point of view. They are also specific against target organisms avoiding to be detrimental towards non-target ones, and their mechanisms of action are less likely to produce resistance by the target organism. Therefore, the identification and the recovery of such bioactive compounds from microalgae is of great interest and their utilization should be supported and encouraged as good practices for plant protection in the context of a sustainable agriculture.

9. Technologies for the production and application of algal bio-fertilizers

Microalgae-based bio-fertilizers should combine the best quality from a nutritional point of view and highly efficient application methods to support an optimal plant development and to maximize crop yields minimizing at the same time losses and environmental contamination (Alvarez *et al.*, 2021). Currently, three main methods to provide bio-fertilizers, including slow-release, N-fixing, and liquid based products are considered and used in agricultural systems. The nutrients required for the generation of algal biomass intended for bio-fertilizer purposes are mainly provided by the preparation of a synthetic standard growth medium and by the recovery of nutrients from the WWs such as WW remediation using Algal Turf Scrubbers (Figure 2),

being the first option characterized by higher costs for the production of algal biomass while the second one is referred to cheaper algal production systems.

Insert here Figure 2

Regardless of the nutrients source open ponds and closed indoor photobioreactors are used to generate algal bio-fertilizers (Mishra and Pabbi, 2004). Among them the recent biofilm-based algal cultivation technology has been reported to be efficient due to its greater harvested biomass production (10-20% in comparison to 0.02% for suspended cultural systems) (Wicker *et al.*, 2023). Moreover, the twin layer (TL) system using WW treatment is applied as an efficient technology due to high growth rate and its nutrients uptake capability, which in turn reduces the cost of the production system through higher biomass productivities (González *et al.*, 2020). Furthermore, scientific evidence shows that algal production systems could be also negatively affected by self-shading phenomena during the photoautotrophic growth, and the risk of contamination can be amplified during a heterotrophic growth owing to the high availability of organic C. However, mixotrophic growth of microalgae appears an ideal approach for the development of technologies aimed at the production of bio-fertilizers by algal biomass (Dagnaisser *et al.*, 2022).

In sustainable agriculture, the N-fixing technology can be effectively used to decrease the N pollution while increasing the yield of crops. In this regard N-fixing cyanobacteria (i.e. *Anabaena* sp, *Tolypothrix* sp., *Scytonema* sp., and *Calothrix* sp.) are recognized as important inputs for the sustainability of crop cultivations and, owing to the wide varieties of their secondary metabolites, they can be simultaneously used as biological pesticides reducing in turn the overall cost of crops production. For example, Bao and coworkers (2021) showed the effectiveness of *Anabaena variabilis* use on the *Oryza sativa* (rice) growth, giving yields by 13.9% - 22% and controlling the rice sheath blight and bacterial blight diseases. Evidence shows that the technology of using N-fixing cyanobacteria improves the soil fertility stabilizing the rapid changes in pH, reduces the usage of chemical fertilizers by 10-15%, increases the availability of organic molecules in the soil, increases the maturity and yield of grain, and protects the plants health from the pathogens (Dhar *et al.*, 2007; Chittora *et al.*, 2020; Sadvakasova *et al.*, 2022).

The technologies of nutrient recovery as bio-fertilizers using algae-based platforms have been introduced as a green alternative and sustainable approach for providing the essential nutrients to

the crops. In this regard, pyrolysis and hydrothermal liquefaction (HTL) of algal biomass attracted great attention due to their efficiency in nutrient recovery. Biochar is a C-enriched compound made through the pyrolysis of organic biomass in the absence of oxygen at high temperature (250-1000 °C). Scientific reports show that biochar obtained by algae such as seaweed have high values in terms of pH, ash and N, contains extractable inorganic molecules (i.e. P, K, Ca, and Mg) and manifests direct nutrient benefit to the acidic soils, even if represents a poor C source (Bird *et al.*, 2011).

HTL of algal biomass is also a promising technology to produce by-products with high concentrations of C, N, P, and growth regulatory compounds that can be used as bio-fertilizers. This technology can be efficiently used for transforming the residual biomass wastes after extraction of oil from the algal biomass during biodiesel production since almost 65% of the residual biomass remains as waste (Caporgno *et al.*, 2016; Djandja *et al.*, 2020). In this regard, it is reported that the use of de-oiled algal biomass as bio-fertilizer had a significant improvement in the fresh weight and yield of *Solanum lycopersicum* by 95% and 174%, respectively (Silambarasan *et al.*, 2021).

Among the fertilizers, P-based chemicals fertilizers (i.e. diammonium phosphate, monoammonium phosphate, and NPKs) are known as important molecules for the growth of crops. However, reports say that ~70% of the used P in agricultural fields is not taken up by the crops and ended up in polluting the ecosystem (Fageria *et al.*, 2013). Currently different technologies, such as struvite recovery and crystallization of phosphate, are being applied for sewage treatment and nutrients recovery in the light of a green approach. Such a process includes the following steps: (1) alga/sewage cultivation (2) HTL of the produced algal biomass (3) recovery of the nutrients from the P-rich solid biochar and N-rich aqueous phase that can be used as fertilizers (Abeyasiriwardana-Arachchige *et al.*, 2021).

A liquid suspension of algal extracts is the most applied method used in the agricultural field. In general, a thermal treatment such as a pasteurization step is used to increase shelf life and avoid spoilage of the liquid agricultural product. These liquid fertilizers are primarily characterized by high trace elements and growth regulatory compounds (particularly cytokinins), which positively impact plant growth and development. In this regard the extracts of algae such as *Chlorella pyrenoidosa*, *Spirulina platensis*, *Acutodesmus dimorphus*, *Microcystis aeruginosa*, *Chlorococcum* sp. have already been commercialized. Algae based bio-fertilizers are also

sprayed on leaves and seeds or directly applied into the soil through the irrigation systems. The application of nutrients through irrigation systems is referred to as nutrification or fertigation which allows an adequate supply of nutrients and water directed towards the plant root zone to satisfy plant demands during the various growth stages. The foliar feeding provides fast, on-the-spot nutrition to ensure top quality yields. It serves as an effective supplementary feeding to complete soil fertilization and for prompt correction of nutrient deficiencies. The application of nutrients on leaves at specific stages of crop development boosts yield and improves plant quality. A recent study carried out by Tejada-Ruiz *et al.* (2020) improved the growth of the ornamental plant *Pelargonium hortum* upon the application of a foliar hydrolysate made of silicon and extracts of *Arthrospira platensis* (containing cytokinins, gibberellins, indoleacetic acid, salicylic acid, and jasmonic acid). On the other hand, soil application is the most used method mainly for open field crops. Bio-fertilizers distributed with these methods demonstrate to be of higher quality if compared to the chemical fertilizers since they contain all the vitamins, phytohormones, amino acids and micro- and macromolecules that are needed for crops development (Zou *et al.*, 2021; Ramya *et al.*, 2015). It has been stated that also the weed control could be accomplished either by the direct spray of algal liquid pesticides or by solid algae plowed into the soil (Singh, 2015). Anyway, it should be noted that today the wide application of algal bio-fertilizers in agriculture worldwide is still challenging (Singh and Vaishnav, 2022).

10. The global market of microalgae-based bio-fertilizers.

It has been recently reported that the global bio-stimulants market had a valued at USD 2.6 billion in 2019 and it is expected to grow 11% through 2025 (Markets and Markets, 2022). Analyses on the fertilizer market demonstrate how the demand for microalgae in this sector is expected to grow at a steady pace, with its overall valuation surpassing US\$ 9479 thousand in 2021, exhibiting a 7.7% year on year growth. Based on experts' opinion, the demand for microalgae, with a CAGR of 5.2% during 2106-2020, is predicted to surge at an 8.7% CAGR over the course of the assessment period, from 2021 to 2031. Growth prospects for the market are likely to remain positive, thanks to improvements in organic farming and the increasing use of bio-fertilizers. *Spirulina* is emerging as a top-selling strain in the fertilizers sector, accounting itself for more than 43% of the global microalgae market in 2021. This strain is followed by *Chlorella*, *Dunaliella*, *Schizochytrium*, *Euglena*, *Nannochloropsis*, and *Nostoc* as

emerging microalgae and cyanobacteria strains on which bio-fertilizers are based (FMI, 2023). France, Germany, China, Japan and USA are currently the top five key producing countries of microalgae in fertilizers sector. In three of these countries are located the top five players such as Algenol, Algatec, Allmicroalgae, Cellana LLC, and Nikken Sohonsa Corp, as shown in **Table 6**. These companies cumulatively were responsible for 55-65% of the total microalgae-based bio-fertilizer market share in 2021.

Insert here Table 6

In terms of the export of microalgae, the fertilizers sector is dominated by the USA, Russia, China, Germany, and Spain. On the other hand, Americans, Germans, French, Chinese, and Japanese represent at the same time the top five users (producers and consumers) which are driving the demand for microalgae in the fertilizers sector. Asian Pacific region displays significant growth opportunities for the market over 2022-2030 owing to a large population of farmers and increasing government initiatives to promote organic farming in the region. At a deep insight, the North American market will be witness to the leading role played by the USA. In 2020 the market of fertilizers from microalgae reached US\$ 2,954 thousand and is projected to total US\$ 6,041 thousand by 2031, growing at a CAGR of 7.4%. These data make the USA the highest consumer of microalgae-based fertilizer in the world. This primacy is mainly due to the rising awareness about environment-friendly agriculture practices and the urge to overcome the problems associated with chemical fertilizer products for long-term opportunities. Moreover, the improved soil stability and the enhanced productivity and quality of crops push the adoption of bio-fertilizers in the American agriculture sector. On the other hand, the Latin American market of microalgae-based fertilizers was dominated by Brazil with a demand of 45.3% in 2021 and is expected to grow at an approximate CAGR of 8.5% throughout the forecast period. The rising demand of microalgal bio-fertilizers in the country was somehow towed by the growing practice of organic food consumption. In European countries the demand for microalgae in bio-fertilizers formulation is mostly driven by the need to reduce abiotic stress which represents constraints to hamper growth and productivity in the Europe agriculture sector. The lion's share is estimated to be hold by Germany through 2031 with a 14.1% by 2031, at a CAGR of 7.6%. An increasingly dominant role will be played by Russia whose demand for microalgae in the fertilizers sector accounted for a value of US\$ 301 thousand in 2021 and is anticipated to total US\$ 934.2

thousand in 2031, with a CAGR of 12.0%. In the Asia Pacific area, the market of bio-fertilizers is dominated by Japan which exhibited a value share of 13.5% in the year 2021 holding the 9th place in the world by volume. The dominant crops in the country are rice and vegetables which cultivation is supported by the Government by fulfilling the raw material procurement in terms of seeds and agrochemicals. The Japanese prefer to improve the quality of food products relying on the massive use of organic farming and bio-fertilizers. In this light, it should be noted the use of *Spirulina* and *Chlorella*-based fertilizers to support the growth of vegetables. Japan's leadership role could soon be undermined by India which undoubtedly represents a key market in Asia for microalgae demand in the fertilizers sector with an approximate volume of around 21.3 dry-weight tons in 2021. This market is predicted to clock a growth rate of 10% in terms of value for 2021-2031 and will contribute over 11% to the total demand for microalgae in fertilizers sector in Asia in 2031. This estimation is based on the relevance that agriculture holds in the economy of the second most populated country in the world. In fact, nearly 8% of total global agriculture output is accounted for in India and the agriculture sector contributed more than 15% to the Indian gross domestic product in 2021. Indian farmers prefer bio-fertilizers to maintain soil fertility in a sustainable manner. Therefore, they are becoming increasingly careful to use fertilizers containing environment-friendly ingredients including microalgae (Aloo *et al.*, 2021, Kumar and Kumar, 2022).

As an index of the worldwide growing interest on bio-fertilizers in less than 10 years there has been a rising trend in the number of patents related to the applications of microalgae in agriculture (Murata *et al.*, 2021). Since the USA, China, and Europe are leaders in microalgae-based agricultural products, it should come as no surprise that they are leaders too in the overall number of patent applications. The annual European Patent Office (EPO) showed that in 2019, about 50% of all patent applications submitted at the EPO came from European companies, followed by American firms for 25% and by companies from Japan, China, and South Korea for lower percentages (EPO, 2020). This aspect can be understood considering that China and European countries contributed in the last years to the global organic farming growth, with a growth of the cultivable available between them from 0.67 to 1 million hectares in 2016. Furthermore, Europe and North America hold the 90% of sales related to organic food consumption. In contrast, regions like Latin America accounts for only 1% of this market (Willer

et al., 2018). Even though the market for organic food in South America is still in its infancy, the growing number of patents in countries such as Brazil, Chile and Peru witness the gradually developing interest for sustainable agriculture.

11. Challenges and perspectives

The issue of crop production is a major challenge today, as global population growth demands a corresponding increase in agriculture output (Frona *et al.*, 2019). Synthetic chemicals such as fungicides, nematicides, and fertilizers are widely exploited to enhance crop production, but their exploitation has been linked to environmental harm and health problems. To decrease the dependence on synthetic chemicals, natural solutions like cyanobacteria and microalgae extracts seem to be promising as they have little or no-negative impact on the environment and human health while supporting sustainable agriculture (Hamed *et al.*, 2018). This complies with the European Regulation EC 1107/2009, which prioritizes non-chemical and natural alternatives whenever possible (EU Regulation 2019/1009, 2019).

The diffusion of bio-compounds for organic farming and in particular of cyanobacteria and microalgae, is still not widespread due to the following main drawbacks:

- *Proper utilization of cyanobacteria and microalgae products.* The advantages of cyanobacteria and microalgae for agriculture can only be pursued through their correct usage. The optimal concentration for promoting plant growth or bio-control is not always the highest. Incorrect dosing can result in negative consequences for plants such as delayed growth, toxicity, or death (Hidangmayum and Sharma, 2017).
- *Scale-up and industrialization.* Despite the promising results obtained at the lab-scale, the number of microalgal bio-products on the agricultural market is still not large. The main challenge for making algal bio-pesticides is to identify the substances with pesticidal properties and how they work (Costa *et al.*, 2019). Another challenge is to optimize their extraction yields by using sustainable extraction agents. Many pesticidal compounds have been discovered but have not yet been commercialized. In fact, most studies on algae focus on biofuels and other bio-products. This makes global policies

crucial in funding researches devoted to investigate the use of algae bio-products in the sustainable agricultural practices of the future.

- *Improving existing standards.* Currently, cyanobacteria and microalgae products often utilize the same types of organisms or by-products. However, the potential of these organisms is vast and bio-diversity is still underexploited. The conventional use of cyanobacteria or microalgae relies on the use of the entire biomass or its extracts. A sustainable solution should also involve the culture supernatant in bio-compounds formulation. Nowadays, after microalgae cultivation, these liquids, which still contain high levels of nutrients and other valuable molecules (Prieto *et al.*, 2011), are often discharged rather than recycled and valued. An instance of successfully applying this concept is represented by the product Naneos (Greencell), a liquid consisting of a mix of *Nannochloropsis oculata* and *Ascophyllum nodosum* extracts.
- *Harmonization of regulations.* Laws on the use of bio-compounds in agriculture vary among countries. From a legislative point of view, a harmonized European Regulation for placing bio-stimulants on the market has been amplified by European countries which globally represent the biggest market for bio-stimulants, with more than 8 million hectares of areas treated throughout 2016 (Liebig *et al.*, 2020). For this reason, the European Community (CE) promulgated for the first time on 2019 an official document (2019/1009), named Fertilizing Products Regulation, which introduced bio-stimulants as fertilizing products allowed to freely circulate inside the CE (EU Regulation 2019/1009, 2019). This Regulation, set to take effect in 2022, will standardize regulations in Europe. This will boost the microalgae market as bio-derived fertilizers will no longer need authorizations, leading to a significant and positive effect on the growth of algae- based biotechnology for agriculture.

Conclusions

Research and development activities focused on cyanobacteria and microalgae plays a crucial role in the future of agriculture. Currently, there are numerous industrial facilities with the ability to produce sufficient quantities to meet agricultural market demand. The number of studies on these organisms continues to grow and the biochemical mechanisms underlying the use of

several species and their extract in agriculture are becoming better understood. However, there is still much to do in order to turn scientific research into practical solutions for industry professionals, even though the market share of these bio-solutions is increasing annually. It is undoubted that one of the advantageous aspect of microalgae-based bio-fertilizers is the possibility to obtain biomass also through the cultivation of microalgae using effluents instead of conventional culture media, potentially reducing process costs and producing microalgae biomass for a variety of uses. In this scenario, microalgae industrial production became feasible owing to the use of reusable resources as a culture medium. In this light, an integrated strategy able to combine CO₂ reduction, WW treatment, and bio-fertilizer production to boost the sustainability and cost-effectiveness of microalgal farming fulfills all the principles of the circular economy.

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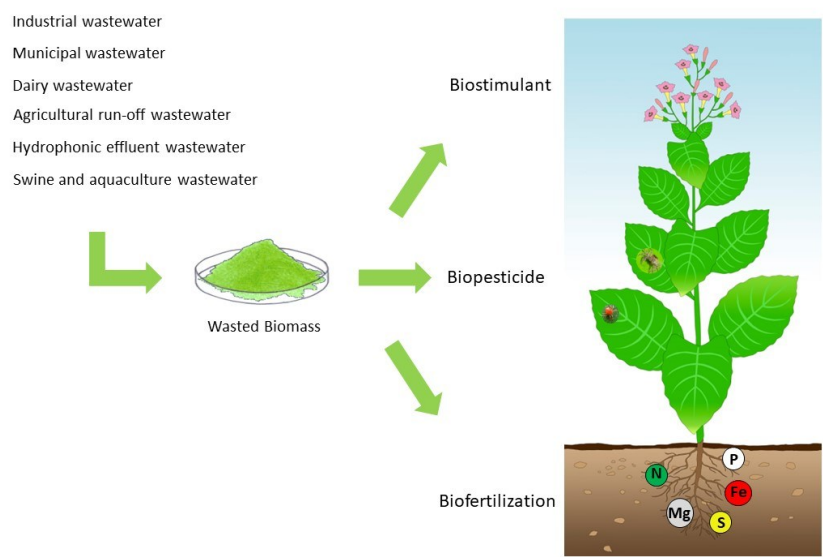


Figure 1. Healthier aspects of algae-based fertilizers application on soil and plants

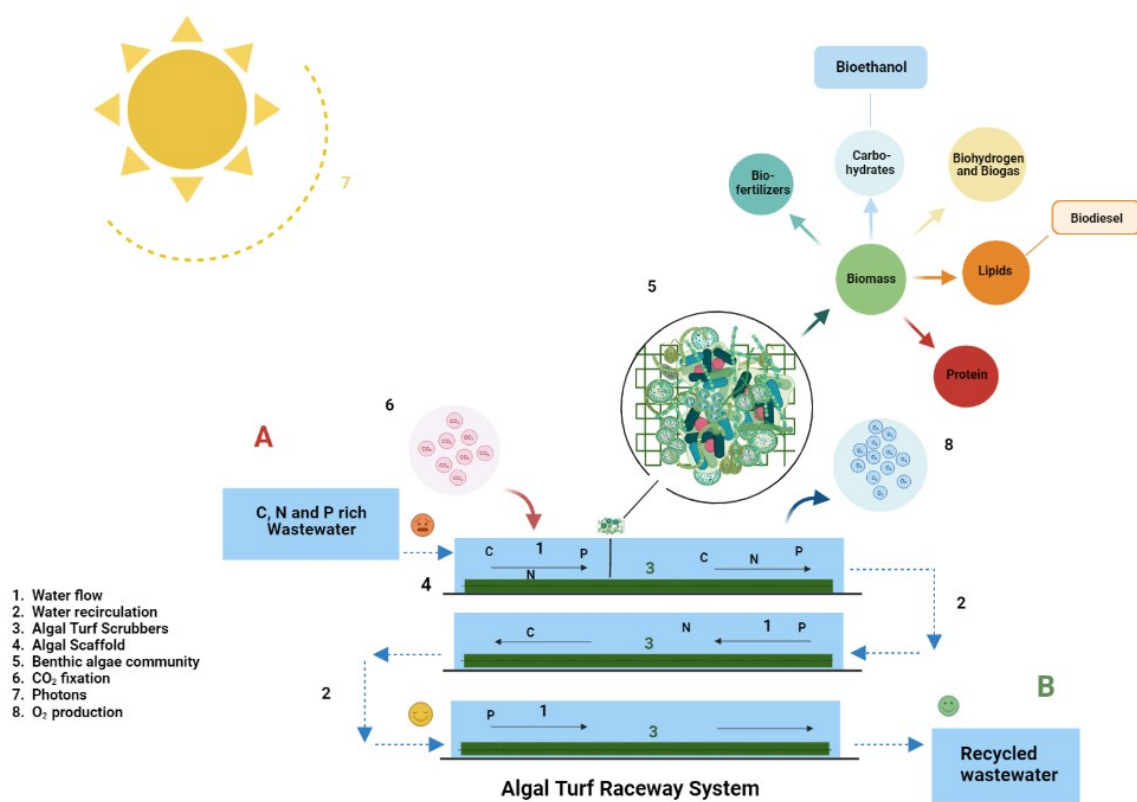


Figure 2. Schematic diagram of the Algal Turf Raceway system for sustainable wastewater management. The excesses amount of C/N and P from the WW systems will be up taken by the introduced Algal Turf Scrubbers platform. The inputs of the system are WW (with excesses amount of C, N, and P) and CO₂, the output will be (1) biomass, (2) recycled WW (3) and O₂. The algal biomass can be used for different purposes including bio-fertilizer and bioenergy production, the recycled water can be reused for the irrigation purposes. In this system the WW flowrate, the population of benthic algae, and the temperature play major roles.

Table 1. Top global bio-fertilizer producing companies

Companies in global regions	Countries	Companies-websites
<i>Europe</i>		
Chr. Hansen Holding A/S	Denmark	https://www.chr-hansen.com/en
Italpollina	Italy	https://www.italpollina.com/
Novozymes	Denmark	https://www.novozymes.com/en
Symborg	Spain	https://symborg.com/es/
Seipasa	Spain	https://www.seipasa.com/it/
Valagro	Italy	https://www.valagro.com/italy/it/
Vegelab SA	Switzerland	https://www.vegalab.com/
<i>North America</i>		
Agrinos	Canada	http://agrinos.com/
Lallemand Inc.	USA	https://www.lallemand.com/
Nutramax Laboratories Inc.	USA	
Valent Bio Science	USA	https://www.valentbiosciences.com/
<i>South America</i>		
Rizobacter Argentina S.A.	Argentina	https://www.rizobacter.com.ar/
<i>Asia</i>		
AgriLife	India	http://agrilife.in/
Aumgene Biosciences	India	https://www.aumgene.com/
Biomax Naturals	India	http://www.biomaxnaturals.com/
Coromandel International Ltd	India	https://www.coromandel.biz/
IPL Biologicals Limited	India	https://www.iplbiologicals.com/
Kan Biosys	India	https://www.kanbiosys.com/
Kiwa Bio-Tech	China	https://kiwabiotech.com/
Jaipur Bio Fertilizers	India	https://www.jaipurbiofertilizers.com/
LKB Bio Fertilizer	Malaysia	https://lkb-group.com/
Manidharma Biotech Pvt Ltd	India	https://www.manidharmabiotech.com/about-us.html
Multiplex Group of Companies	India	https://www.multiplexgroup.com/
T. Stanes & Company Limited	India	https://tstanes.com/
The Fertilisers and Chemical Travancore Ltd	India	https://www.fact.co.in/
UPL Limited	India	https://www.upl-ltd.com/
Varsha Bioscience and Technology	India	https://www.varshabioscience.com/
India Pvt Ltd.		
<i>Oceania</i>		
Mapleton Agri Biotech Pty Ltd	Australia	https://www.mabiotec.com/

Table 2. Efficiency of agriculture related wastewater treatment by microalgae and cyanobacteria.

Wastewater	Strain	Removal efficiency (%)			Biomass (g L ⁻¹ or g m ²)	References
		COD	TN	TP		
Aquaculture	<i>Monoraphidium</i> sp.;	NA	82-98	66-90	0.6-1.2	Guerrero-Cabrera <i>et al.</i> , 2014
	<i>Scenedesmus</i> sp.					
	<i>Chlorella sorokiniana</i>	72	75.5-84.5	73.3	1.15-4	Guidhe <i>et al.</i> , 2017
	<i>Chlorella sorokiniana</i>	NA	86.4	NA	1.93	He <i>et al.</i> , 2023
	<i>Chlorella vulgaris</i>	NA	79	94	1.39	Tejido-Nunez <i>et al.</i> , 2019
Agricultural runoff	<i>Chlorella vulgaris</i>	75.5	76.5	93	1.31	Daneshvar <i>et al.</i> , 2018
	<i>Chlorella vulgaris</i>	NA	93.5	92.7	~0.45	Daneshvar <i>et al.</i> , 2018
	Cyanobacteria	NA	95	99	NA	Rueda <i>et al.</i> , 2020
	<i>Anabaena sphaerica</i> ,	77-84	~90	~50	~1.1	Gorain <i>et al.</i> , 2019
	<i>A. Variabilis</i>					
	<i>Chlorella</i> sp.,		86	80	0.9	Castellano-Eastupinas <i>et al.</i> , 2022
	<i>Scenedesmus</i> sp.,		88	81	0.8	Castellano-Eastupinas <i>et al.</i> , 2022
	<i>Hapalosyphon</i> sp.		85	80	0.7	Castellano-Eastupinas <i>et al.</i> , 2022
	Microalgae community	NA	80-90	66-91	227.6	Singh and Patidar, 2023
	<i>Chlorella</i> sp.	NA	100	89	2.4	Cowan <i>et al.</i> , 2022
Hydroponic effluent	<i>Scenedesmus</i> sp.	NA	35	69	1.07	Salazar <i>et al.</i> , 2021
	<i>Chlorella vulgaris</i>	NA	90	99	1.26	Yousif <i>et al.</i> , 2022
	<i>Chlorella vulgaris</i>	NA	85.6	96	4.5	Varirzadeh <i>et al.</i> , 2022
	<i>Chlorella</i> sp.	29.5-44	49.5-51	45-54.5	1.40-1.54	Lu <i>et al.</i> , 2015
Animal farm meat processq	<i>Scenedesmus</i> sp.	65	78	78	NA	Ahmad Latiffi <i>et al.</i> , 2022
	<i>Scenedesmus dimorphus</i>	NA	90	65	1.1	Danee <i>et al.</i> , 2022
	<i>Scenedesmus</i> sp.	>90	>90	79-88	2-4	Luo <i>et al.</i> , 2019
Cattle	<i>Kirchneriella obesa</i>	85.8	99.6	99.1	1.14	Wang <i>et al.</i> , 2021
Swine	<i>Chlorella pyrenoidosa</i>	57.3	33.7	61.9	0.99	Wu <i>et al.</i> , 2019
	<i>Chlorella vulgaris</i>	95.7	69.5	64.5	7.4	Wu <i>et al.</i> , 2019
	<i>Picochlorum oklahomensis</i>	7	100	85	2.7	Lutzu <i>et al.</i> , 2020
	<i>Tribonema</i> sp.	50-55.5	80-99	66.5-73	~1.4	Cheng <i>et al.</i> , 2020
	<i>Synechocystis</i> sp.	63-68.5	74-76	71.4-71.6	~1.6	Cheng <i>et al.</i> , 2020
Food Industry Brewery	<i>Scenedesmus dimorphus</i>	65	>99	>99	6.82	Lutzu <i>et al.</i> , 2016
	<i>Leptolyngbya</i> sp.	91.6	89.4	98.5	0.74	Papdopulos <i>et al.</i> , 2020
	<i>Chlamydomonas reinhardtii</i>	43.8	86.2	73.6	NA	Su <i>et al.</i> , 2022
	<i>Chlorella vulgaris</i>	46.0	94.8	77.0	NA	Su <i>et al.</i> , 2022

Dairy	<i>Scenedesmus obliquus</i>	44.3	70.7	75.6	NA	Su <i>et al.</i> , 2022
	Wastewater <i>born</i>	49.3	84.4	71.7	NA	Kumar <i>et al.</i> , 2019
	<i>microalgae</i>					
	<i>Ascochloris</i> sp.	94-96	72-80	80-97	2	Kumar <i>et al.</i> , 2019
	<i>Auxenochlorella</i>	78	69	35	3	Gramegna <i>et al.</i> , 2020
	<i>prototechoides</i>					
	<i>Chlorella</i> sp.	27-38	76-82.5	62.5-75	1.47-1.71	Wang <i>et al.</i> , 2010
	<i>Chlorella sorokiniana</i>	91.3	96.3	92.1	4.8	Kusmayadi <i>et al.</i> , 2022
	<i>Chlorella vulgaris</i>	16.5-45.5	42-6.65	21.8-64	NA	Lu <i>et al.</i> , 2015
	<i>Chlorella vulgaris</i>	46.7	82.8	99.6	5.4	Zhu <i>et al.</i> , 2022
	<i>Chlamydomonas</i>	78	70	41	1.9	Gramegna <i>et al.</i> , 2020
	<i>reinhardtii</i>					
	<i>Euglena gracilis</i>	91	90	85	3.2	Barasanti <i>et al.</i> , 2020
	<i>Scenedesmus</i>	77	92	100	0.43	Daneshvar <i>et al.</i> , 2019
	<i>quadricauda</i>					

NA: not available

Table 3. Application of microalgae as bio-fertilizers (adapted from La Bella *et al.*, 2022)

Microalgae	From	Traget crops	Effects	Reference
Anabaena sp.	Cellular extract, biomass	<i>Salix viminalis</i> (Willon)	C, D, E, H	Grzesik <i>et al.</i> , 2017
<i>Anabaena variabilis</i>	Living cells	<i>Oryza</i> sp. (rice)	B, H	EI-Sheekh <i>et al.</i> , 2018
<i>Anabaena</i> , <i>Fischerella</i> , <i>Phormidium</i> , <i>Spirogyra</i> , <i>Westiellopsis</i> as consortium	Biomass	<i>Triticum aestivum</i> (wheat)	E, F	Renuka <i>et al.</i> , 2016
<i>Anabaena</i> , <i>Anabaenopsis</i> , <i>Aulosira</i> , <i>Aphanotece</i> , <i>Calhotrix</i> , <i>Camptylonema</i> , <i>Cylindrospermum</i> , <i>Chlorogleopsis</i> , <i>Camptilonema</i> , <i>Chlorogloea</i> , <i>Fischerella</i> , <i>Nostoc</i> , <i>Nodularia</i> , <i>Nostochopsis</i> , <i>Wolleea</i> , <i>Scytonema</i> , <i>Mastigocladus</i> , <i>Stigonema</i> , <i>Rivularia</i> , <i>Westiella</i> , <i>Westiellopsis</i> , <i>Plectonema</i> , <i>Haplisiphon</i> , <i>Schytoneumatopsis</i> , <i>Gloeotrichia</i> <i>Arthrospira platensis</i>	--	<i>Oryza</i> sp. (rice)	E, H, I	Watanabe and Cholikul, 1979; Vaishampayan, 1998, Singh, 1961
	Biomass	<i>Eruca sativa</i> (arugula), <i>Amaranthus gangeticus</i> (Bayam Red), <i>Brassica rapa</i> spp. <i>chinensis</i> (Pak Choi), <i>Brassica oleracea alboglabra</i> (Kai Lan)	A, H	Wuang <i>et al.</i> , 2016
<i>Arthrospira platensis</i>	Biomass	<i>Oryza</i> sp. (rice)	D, E, F	Dineshkumar <i>et al.</i> , 2018a
<i>Arthrospira platensis</i>	Biomass	<i>Triticum aestivum</i> (wheat)	E, F	Alobwede <i>et al.</i> , 2019
<i>Arthrospira platensis</i>	Solution	<i>Apium graveolens</i> (celery)	H	Rashad <i>et al.</i> , 2019
<i>Chlorella minutissima</i>	Biomass	<i>Spinacia oleracea</i> (spinach)	E, F	Sharma <i>et al.</i> , 2021
<i>Chlorella minutissima</i>	Biomass	<i>Lactuca sativa</i> (lettuce)	A, B, C	Elhafiz <i>et al.</i> , 2015
<i>Chlorella pyrenoidosa</i>	Solution	<i>Lactuca sativa</i> (lettuce), <i>Oryza</i> sp. (rice), <i>Solanum melongena</i> (aubergine), <i>Cucumis sativus</i> (cucumber)	A, B, C	Elhafiz <i>et al.</i> , 2015
<i>Chlorella</i> sp.	Biomass	<i>Triticum aestivum</i> (wheat)	E, F	Alobwede <i>et al.</i> , 2019
<i>Chlorella</i> sp.	Cellular extract, biomass	<i>Salix viminalis</i> (willow)	C, D, E, H	Grzesik <i>et al.</i> , 2017
<i>Chlorella</i> , <i>Chlorococcum</i> , <i>Chroococcus</i> , <i>Scenedesmus</i> as consortium	Biomass	<i>Triticum aestivum</i> (wheat)	E, F	Renuka <i>et al.</i> , 2016
<i>Chlorella vulgaris</i>	Solution	<i>Vitis</i> L. (grapevines)	E, G	Abd EI Moniem <i>et al.</i> , 2008
<i>Chlorella vulgaris</i>	Biomass	<i>Oryza</i> sp. (rice)	D, E, F	Dineshkumar <i>et al.</i> , 20178a
<i>Chlorella vulgaris</i>	Cellular extracts	<i>Vigna mungo</i> (black gram)	E, F	Dineshkumar <i>et al.</i> , 20178c
<i>Chlorella vulgaris</i>	Biomass	Application on soil	E, F	Gougoulas <i>et al.</i> , 2018

<i>Chlorella vulgaris</i>	Cellular extracts, living cells	Application on soil	E, F	Barone <i>et al.</i> , 2019
<i>Chlorella vulgaris</i>	Solution	<i>Solanum lycopersicum</i>	E, F	Suchitra <i>et al.</i> , 2022
<i>Chlorella vulgaris</i> ; <i>Arthrospira platensis</i>	Solution	<i>Vigna radiata</i> (mung bean)	C, E, H, I	Dineshkumar <i>et al.</i> , 2022
<i>Chlorella pyrenoidosa</i>	Living cells	<i>Chenopodium quinoa</i> (quinoa)	A, B, F, H	Ma <i>et al.</i> , 2022
<i>Chlorella vulgaris</i> ; <i>Scenedesmus obliquus</i>	Living cells	<i>Lactuca sativa</i> (lattice)	B, E, H	Alvarenga <i>et al.</i> , 2022
Microalgae bacteria flocs	Biomass	<i>Solanum lycopersicum</i> (tomato)	G	Coppens <i>et al.</i> , 2016
<i>Mycrocistis aeruginosa</i>	Cellular extract,biomass	<i>Salix viminalis</i> (willow)	C, D, E, H	Grzesik <i>et al.</i> , 2017
<i>Nannochloropsis</i> sp.	Biomass	<i>Solanum lycopersicum</i> (tomato)	G	Coppens <i>et al.</i> , 2016
<i>Nostoc</i> sp.	Biomass	<i>Capsicum annum</i> (pepper)	E, H	Singh, 2017
<i>Nostoc calcicola</i> , <i>N. Linkia</i>	Living cells	<i>Oryza</i> sp. (rice)	B, H	EI-Sheek <i>et al.</i> , 2018
<i>Scenedesmus</i> sp.	Biomass	<i>Ocimum basilicum</i> (basil)	C, D, H	Alvarez-Gonzalez <i>et al.</i> , 2022
<i>Scenedesmus quadricauda</i>	Cellular extracts, living cells	Appliation on soil		
<i>Scytonema hofmanni</i>	Cellular extracts	<i>Oryza</i> sp. (rice)	B	Rodriguez <i>et al.</i> , 2006
VZ Biofertilizer (microalgae), <i>TTB (Bacillus)</i>	Living cells	<i>Salvia miltiorrhiza</i> (red sage)	D, F, H, I	Wei <i>et al.</i> , 2023

A = improving germination process, B = improving salinity tolerance, C = enhancing chlorophyll content, D = improving biological activity of the soil, E = improving chemical properties of the soil, F = increasing the availability of macronutrients, G = improving fruit quality, H = enhancing plant growth, I = producing growth promoting substances

Table 4. Application of microalgae as bio-stimulants (adapted from La Bella *et al.*, 2022)

Microalgae	Extract/Biomass	Application	Effects	Reference
<i>Acutodesmus dimorphus</i>	Cellular extracts, growth medium and culture	<i>Solanum lycopersicum</i> cv Roma (tomato)	A, B	Garcia-Gonzalez and Sommerfeld, 2016
<i>Anabaena</i> sp.	Cellular extract, biomass	<i>Salix viminalis</i> (willow)	B,C	Grzesik <i>et al.</i> , 2017
<i>Anabaena vaginicola</i> ISC90	Algal extracts	<i>Cucurbita maxima</i> (squash), <i>Cucumis sativus</i> (cucumber), <i>Solanum lycopersicum</i> (tomato)	E, F,Q,R	Shariatmadari <i>et al.</i> , 2013
<i>Anabaena</i> , <i>Fisherella</i> , <i>Phormidium</i> , <i>Spirogyra</i> , <i>Westiellopsis</i> as consortium	Biomass	<i>Triticum aestivum</i> (wheat)	E	Renuka <i>et al.</i> , 2016
<i>Arthrospira maxima</i> , <i>Arthrospira plantensis</i>	Crude bioextracts (CBEs)	<i>Solanum lycopersicum</i> (tomato)	C, D, E, F	Mutale-Joan <i>et al.</i> , 2020
<i>Arthrospira plantensis</i>	Hydrolysate extracts	<i>Petunia hybrida</i> (petunia)	E, G, H, I	Plaza <i>et al.</i> , 2018
<i>Arthrospira plantensis</i>	Algal extracts	<i>Phaseolus vulgaris</i> (common bean)	B,C,I,M,N, O, P	Rady <i>et al.</i> , 2023
<i>Arthrospira plantensis</i> , <i>Nostoc muscorum</i> , <i>Anabaena oryzae</i>	Living cells suspension	<i>Apium graveolens</i> (celery)	E, K, L	Rashad <i>et al.</i> , 2019
<i>Arthrospira plantensis</i> plus cow dung	Biomass	<i>Allium sativa</i> (onion)	A, E, I, L	Dineshkumar <i>et al.</i> , 2018b
<i>Aphanotece</i> sp.,	CBEs	<i>Solanum lycopersicum</i> (tomato)	C, D, E, F	Mutale-Joan <i>et al.</i> , 2020
<i>Chlorella</i> , <i>Chlorococcum</i> , <i>Chroococcus</i> , <i>Scenedesmus</i> as consortium	Biomass	<i>Triticum aestivum</i> (wheat)	E	Renuka <i>et al.</i> , 2016
<i>Chlorella</i> , <i>C. Vulgaris</i> , <i>Scenedesmus acutus</i>	Extracts	<i>Vigna radiata</i> (mung bean)	E	Stirk <i>et al.</i> , 2020
<i>Chlorella</i> sp.	Cellular extract, biomass	<i>Salix viminalis</i> (Willow)	B, C	Grzesik <i>et al.</i> , 2017
<i>Chlorella ellipsoidale</i> ; <i>Chlorella marina</i>	CBEs,	<i>Solanum lycopersicum</i> (tomato)	C, D, E, F	Mutale-Joan <i>et al.</i> , 2020
<i>Chlorella minutissima</i>	Biomass	<i>Spinacia oleracea</i> (spinach), <i>Zea mays</i> (baby corn)	E, O	Sharma <i>et al.</i> , 2021
<i>Chlorella prototechoides</i>	Living cells suspension	<i>Cucumis sativus</i> (cucumber), <i>Hordeum vulgare</i> (barley), <i>Triticum aestivum</i> (wheat), <i>Glycine max</i> (soybean), <i>Nasturtium officinale</i> (watercress), <i>Solanum lycopersicum</i> (tomato)	A, C, E, F, M	Ferreira <i>et al.</i> , 2021
<i>Chlorella pyreïnoidosa</i>	Biomass	<i>Abelmoschus angulosus</i> (okra)	C, E, F	Umamhesvari and Shanthakumar, 2020
<i>Chlorella sorokiniana</i>	CBEs	<i>Solanum lycopersicum</i> (tomato)	C, D, E, F	Mutale-Joan <i>et al.</i> , 2020
<i>Chlorella vulgaris</i>	Cellular extracts	<i>Beta vulgaris</i> cv Shannon (beet)	J, K	Puglisi <i>et al.</i> , 2020a
<i>Chlorella vulgaris</i>	Cellular extracts	Lettuce seedlings	C, L, M, N,	Puglisi <i>et al.</i> , 2022

			O	
<i>Chlorella vulgaris</i>	Algal extracts	<i>Lactuca sativa</i> (lattice)	B,C, L, M, N	Puglisi <i>et al.</i> , 2022
<i>Chlorella vulgaris</i>	Cellular extracts	<i>Vigna mungo</i> (black gram)	E, F	Dineshkumar <i>et al.</i> , 2018c
<i>Chlorella vulgaris</i>	CBEs	<i>Solanum lycopersicum</i> (tomato)	J, K	Mutale-Joan <i>et al.</i> , 2020
<i>Chlorella vulgaris</i>	Living cells suspension	<i>Solanum lycopersicum</i> (tomato)	A, J	Bumandalai and Tserennadmid, 2019
<i>Chlorella vulgaris</i>	Living cells suspension	<i>Solanum lycopersicum</i> (tomato)	B, P	Suchithra <i>et al.</i> , 2022
<i>Chlorella vulgaris</i>	Algal extracts	<i>Pelargonium</i> × <i>hortorum</i>	H, Q, R	Bayona-Morcillo <i>et al.</i> , 2022
<i>Chlorella vulgaris</i>	Living cells suspension	<i>Cucumis sativus</i> (cucumber)	A, J	Bumandalai and Tserennadmid, 2019
<i>Chlorella vulgaris</i>	Living cells suspension	<i>Cucumis sativus</i> (cucumber), <i>Hordeum vulgare</i> (barley), <i>Triticum aestivum</i> (wheat), <i>Glycine max</i> (soybean), <i>Nasturtium officinale</i> (watercress), <i>Solanum lycopersicum</i> (tomato)	A, C, E, F, M	Ferreira <i>et al.</i> , 2021
<i>Chlorella vulgaris</i> , <i>Scenedesmus quadricauda</i>	Algal extracts	<i>Lactuca sativa</i> (lattice)	C, M, N, O	Santoro <i>et al.</i> , 2022
<i>Chlorella vulgaris</i> plus Cow dung	Biomass	<i>Allium sativa</i> (Onion)	A, E, I, L	Dineshkumar <i>et al.</i> , 2018b
<i>Chlorella vulgaris</i> , <i>Chlorella</i> sp., <i>Chlamydomodium fusiforme</i>	Biomass	<i>Lepidium sativum</i> (watercress), <i>Glycine max</i> (soy), <i>Cucumis sativus</i> (cucumber), <i>Triticum aestivum</i> (wheat)	A,C,K,J	Morillas-Espana <i>et al.</i> , 2022
<i>Clamidomonas reinhardtii</i> , <i>Dunaliella salina</i> , <i>Isochrysis galbana</i> , <i>Mycrocistis aeruginosa</i>	CBEs	<i>Solanum lycopersicum</i> (tomato)	C, D, E, F	Mutale-Joan <i>et al.</i> , 2020
	Cellular extract, biomass	<i>Salix viminalis</i> (willow)	B,C	Grzesik <i>et al.</i> , 2017
<i>Nannochloropsis gaditana</i>	CBEs	<i>Solanum lycopersicum</i> (tomato)	C, D, E, F	Mutale-Joan <i>et al.</i> , 2020
<i>Nostoc calcicola</i> ISC90	Algal extracts	<i>Cucurbita maxima</i> (squash), <i>Cucumis sativus</i> (cucumber), <i>Solanum lycopersicum</i> (tomato)	E, F, Q, R	Shariatmadari <i>et al.</i> , 2013
<i>Nostoc carneum</i> ISB88, <i>Nostoc punctiforme</i> ISB90	Living cells suspension	<i>Matricaria camomilla</i> (chamomile)	E, F	Zarezadeh <i>et al.</i> , 2020
<i>Nostoc entophyllum</i> ; <i>Oscillatoria angustissima</i>	Living cells suspension	<i>Pisum sativa</i> (pea)	A, E, F	Osman <i>et al.</i> , 2020
<i>Phaeodactylum tricornutum</i>	Biomass	<i>Lepidium sativum</i> (watercress)	A, J	Ennis <i>et al.</i> , 2017
<i>Porphyridium</i> sp.	CBEs	<i>Solanum lycopersicum</i> (tomato)	C, D, E, F	Mutale-Joan <i>et al.</i> , 2020
<i>Scenedesmus almeriensis</i>	Hydrolystate extracts	<i>Petunia hybrida</i> (petunia)	E, G, H, I	Plaza <i>et al.</i> , 2018
<i>Scenedesmus dimorphus</i> , <i>Scenedesmus obliquus</i>	CBEs	<i>Solanum lycopersicum</i> (tomato)	C, D, E, F	Mutale-Joan <i>et al.</i> , 2020
<i>Scenedesmus obliquus</i>	Biomass	<i>Lepidium sativum</i> (watercress)	A, J	Navarro-Lopez <i>et al.</i> , 2020

<i>Scenedesmus obliquus</i>	Biomass	<i>Vigna radiata</i> (mung bean), <i>Cucumis sativus</i> (cucumber)	E	Navarro-Lopez <i>et al.</i> , 2020
<i>Scenedesmus quadricauda</i>	Cellular extracts	<i>Beta vulgaris</i> cv <i>Shannon</i> (beet)	J, K	Puglisi <i>et al.</i> , 2020a
<i>Scenedesmus quadricauda</i>	Cellular extracts	<i>Lettuce seedlings</i>	C,L,M,N,O	Puglisi <i>et al.</i> , 202b
<i>Tetradesmus obliquus</i>	Living cells suspension	<i>Cucumis sativus</i> (cucumber), <i>Hordeum vulgare</i> (barley), <i>Triticum aestivum</i> (wheat), <i>Glycine max</i> (soybean), <i>Nasturtium officinale</i> (watercass), <i>Solanum lycopersicum</i> (tomato)	A, C, E, F, M	Ferreira <i>et al.</i> , 2021
<i>Tetraselmis marina</i> , <i>Tetraselmis suecica</i> , <i>Tetraselmis</i> sp.	CBEs	<i>Solanum lycopersicum</i> (tomato)	C, D, E, F	Mutale-Joan <i>et al.</i> , 2020
<i>Wolleea vaginicola</i> ISB89	Living cells suspension	<i>Matricaria camomilla</i> (chamomile)	E, F	Zarezaadeh <i>et al.</i> , 2020

A = improving seed germination, B = increasing plant growth by foliar application, C = improving chlorophyll content, D = improving nutrient uptake. E = improving root length and dry weight, F = improving shoot length and dry weight, G = improving flower dry weight, H = improving flower fresh weight, I = improving plant nutrition status, J = improving germination rates, K = improving root development, L = increasing dry matter, M = increasing carotenoids, N = increasing proteins, O = influencing the activity of enzymes, P = improving nutritional content of fruits, Q = improving plant height, R = improving number of leaves

Table 5. Application of microalgae-cyanobacteria as bio-pesticides (adapted from La Bella et al. 2022)

Microalgae	Application	Target pathogen	Protected plant	Effects	Reference
<i>Anabaena laxa</i> RPN8	<i>In vivo</i>	<i>Rhizoctonia</i> sp. ^d	--	A, B, C	Babu <i>et al.</i> , 2015
<i>Anabaena oryzae</i>	Extracts	<i>Meloydogine incognita</i> ^f	Banana	I	Hamouda and El-Anasry, 2017
<i>Anabaena</i> sp.	Aqueous extracts	<i>Botrytis cinerea</i> ^d	Strawberry	I	Righini <i>et al.</i> , 2019
<i>Arthrospira</i> sp.	<i>In vitro</i>	<i>Fusarium graminearum</i> ^d	Vine	E, F, G, H	Scaglioni <i>et al.</i> , 2019
<i>Arthrospira</i> sp.	Ethanol extracts	<i>Spodoptera littoralis</i> ^g	Cotton, tomato, maize	I	Rashwan <i>et al.</i> , 2020
<i>Arthrospira platensis</i>	Extracts	<i>Alternaria alternata</i> , <i>Botrytis cinerea</i> , <i>Rhizoctonia solani</i> , <i>Sclerotium rolfsii</i> ^d	--	I	Schmid <i>et al.</i> , 2022
<i>Arthrospira platensis</i> , <i>Arthrospira maxima</i>	Extracts	<i>Dermatophagoides pteronyssinus</i> ^g	--	I	Ruangsomboon, 2016
<i>Calotrix</i> sp.	<i>In vitro</i>	<i>Rhizoctonia</i> sp. ^d	--	A, B, C,	Babu <i>et al.</i> , 2015
<i>Chlorella prototechoides</i> ^c	<i>In vivo</i>	<i>Fusarium oxysporum</i> ^d	--	I	Ferreira <i>et al.</i> , 2021
<i>Chlorella sorokiniana</i>	Extracts	<i>Phytophthora cactorum</i> ^d	Strawberry	I	Jokel <i>et al.</i> , 2023
<i>Chlorella vulgaris</i>	Extracts	<i>Meloydogine incognita</i> ^f	Banana	I	Hamouda and El-Anasry, 2017
<i>Chlorella vulgaris</i>	<i>In vivo</i>	--	Maize	C, I	Goncalves, 2021
<i>Chlorella vulgaris</i>	Powder	<i>Xiphinema index</i> ^f	Grapevine	I	Bileva, 2013
<i>Chlorella vulgaris</i>	Extracts	<i>Alternaria alternata</i> , <i>Botrytis cinerea</i> , <i>Rhizoctonia solani</i> , <i>Sclerotium rolfsii</i> ^d	--	I	Schmid <i>et al.</i> , 2022
<i>Chlorella vulgaris</i> ^c	<i>In vivo</i>	<i>Fusarium oxysporum</i> ^d	--	I	Ferreira <i>et al.</i> , 2021
<i>Chlorella vulgaris</i> MACC-1 ^a	<i>In vitro</i>	<i>Fusarium oxysporum</i> sp. <i>melonis</i> ^d , <i>Rhizoctonia solani</i> ^d	--	D	Ranglova <i>et al.</i> , 2021
<i>Chlorella vulgaris</i> MACC-1 ^a	<i>In vitro</i>	<i>Phytophthora capsici</i> ^d	Pepper	D	Ranglova <i>et al.</i> , 2021
<i>Chlorella vulgaris</i> MACC-1 ^a	<i>In vitro</i>	<i>Clavibacter michiganensis</i> sp. <i>michiganensis</i> ^d	Tomato, Potato	D	Ranglova <i>et al.</i> , 2021
<i>Chlorella vulgaris</i>	<i>In vitro</i>	<i>Fusarium oxysporum</i> sp.	--	D	Ranglová <i>et al.</i> , 2021

MACC-1 ^b		<i>melonis</i> ^d , <i>Rhizoctonia solani</i> ^d , <i>Phytophthora capsici</i> ^d , <i>Phytium ultimum</i> ^d , <i>Clavibacter michiganensis</i> sp. <i>michiganensis</i> ^d , <i>Pseudomonas syringae</i> pv., <i>Pectobacterium carotovorum</i> ^e			
<i>Chlorella vulgaris</i> MACC-1 ^b	In vitro	<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> ^e	Crucifer crops	D	Ranglová <i>et al.</i> , 2021
<i>Cyanosarcina fontana</i>	Powder	<i>Meloydogine incognita</i> ^f	Plants	I	El-Deen and Issa, 2016
<i>Leptolyngbya</i> sp.	Extract	<i>Clavibacter michiganensis</i>	Tomato	A, C, I	Toribio <i>et al.</i> , 2021
<i>Scenedesmus</i> sp.					
<i>Nannochloropsis</i> sp.	In vitro	<i>Fusarium graminearum</i> ^d	--	E, F, G, H	Scaglioni <i>et al.</i> , 2019
<i>Nannochloropsis</i> sp.	Extracts	<i>Alternaria alternata</i> , <i>Botrytis cinerea</i> , <i>Rhizoctonia solani</i> , <i>Sclerotium rolfsii</i> ^d	--	I	Schmid <i>et al.</i> , 2022
<i>Nostoc</i> sp.	In vivo	--	Pepper	I	Singh <i>et al.</i> , 2017
<i>Nostoc</i> sp.	In vitro	<i>Sclerotinia sclerotinium</i> ^d	Tomato	I	Biondi <i>et al.</i> , 2004
<i>Nostoc</i> sp.	Ethanol extracts	<i>Armillaria</i> sp., <i>Fusarium oxysporum</i> f. sp. <i>melonis</i> , <i>Penicillium expansum</i> , <i>Phytophthora cambivora</i> , <i>P. cinnamomi</i> , <i>Rhizoctonia solani</i> , <i>Rosellinia</i> sp., <i>Verticillium albo-atrum</i> ^d	Plants	I	Bondi <i>et al.</i> , 2004
<i>Nostoc</i> sp.	In vitro	<i>Caenorhabditis elegans</i> ^f	Plants	I	Biondi <i>et al.</i> , 2004
<i>Nostoc carneum</i> , <i>Parachlorella kessleri</i>	Extracts	<i>Spodoptera littoralis</i> ^g	Cotton	I	Saber <i>et al.</i> , 2018
<i>Oscillatoria</i> sp.	Extracts	<i>Dermatophagoides pteronyssinus</i> ^g	--	I	Ruangsomboon, 2016
<i>Phaeodactylum tricornutum</i>	Extracts	<i>Alternaria alternata</i> , <i>Botrytis cinerea</i> , <i>Rhizoctonia solani</i> , <i>Sclerotium rolfsii</i> ^d	--	I	Schmid <i>et al.</i> , 2022
<i>Phormidium</i> sp.	Extracts	<i>Dermatophagoides pteronyssinus</i> ^g	--	I	Ruangsomboon, 2016
<i>Scenedesmus obliquus</i>	Powder	<i>Meloydogine incognita</i> ^f	Plants	I	El-Deen and Issa, 2016
<i>Scenedesmus obliquus</i>	Extracts	<i>Meloydogine incognita</i> ^f	Banana	I	Hamouda and El-Anasry, 2017

<i>Scenedesmus obliquus</i>	Extracts	<i>Alternaria alternata</i> , <i>Botrytis cinerea</i> , <i>Rhizoctonia solani</i> , <i>Sclerotium rolfsii</i> ^d	--	I	Schmid <i>et al.</i> , 2022
<i>Scytonema</i> MKU106	Extracts	<i>Sylepta derogata</i> ^g	Cotton	I	Sathiyamoorthy and Shanmugasundaram, 1996
<i>Tetrademus obliquus</i> ^c	<i>In vivo</i>	<i>Fusarium oxysporum</i> ^d	--	I	Ferreira <i>et al.</i> , 2021

^acultivated in BG-11, ^bcultivated in urban wastewater, ^ccultivated in piggery wastewater, ^dfungus, ^ebacterium, ^fnematode, ^ginsect; A = enhancing the levels of defensive enzymes, B = reducing mortality, C = improving growth and yield, D = inhibiting development, E = reducing mycelia halo formation, F = reducing ergosterol production, G = inhibiting acetylates production, H = inhibiting trichothecenes production, I = protection against pathogens, viruses, or other biotic or abiotic stress conditions

Table 6. Microalgae-based bio-fertilizer companies

Name of the Company	Country	Company website
Algenol	USA	https://www.algenol.com/
Algatec	Portugal	https://a4f.pt/en/projects/algatec
Allmicroalgae	Portugal	https://www.allmicroalgae.com/en/
Aqualgae	Spain, Portugal	https://aqualgae.com/es/empresa/
Cellana LCC	USA	http://cellana.com/
Nikken Sohonsa Corp	China	https://www.nikken-miho.com/
Haifa Group	Israel	https://www.haifa-group.com/about-us-0
Terra Aquatica T.A.	France	https://www.terraaquatica.com/fr/
Qingdao Hibong Fertilizer Co., Ltd.	China	http://fundesk.net/index.html@p=%252FD0%252Farea&lg=en.html
AlgaEnergy	Spain	https://ag.algaenergy.com/
Panpan Industry	China	https://panpanindustry.en.china.cn/
TrueAlgae	USA	https://truealgae.com/
New-Nutri	China	https://www.new-nutri.com/
Hispanagar S A	Spain	https://www.hispanagar.com/en/company
Algea	Norway	https://www.algea.com/
Growtent	Poland	https://www.growtent.eu/eng_m_LINKS_Algae-based-fertilizers-2808.html
Heliae	USA	https://heliae.com/about/
Algamar	Mexico	https://grupoalgamar.com/division/agriculture/
BOC Sciences	USA	https://bio-fermen.bocsci.com/
BDI biotech	Spain	https://bdi.biotech.com/
CARTIF	Spain	https://www.cartif.es/en/about-us/
Envii	UK	https://www.envii.co.uk/
Growland	Germany	https://www.growland.net/
Enlasa	Guatemala	https://grupoenlasa.com/algatec/
TerraSync – Accellergy Corp	USA	https://www.accelergy.co/
Enlightened Soil Corp	USA	https://www.enlightenedsoil.com/
Plymag	Spain	https://www.plymag.com/en/fertilizer-manufacturing-companies/
Algaenergy	Spain	https://www.algaenergy.com/
Biorizon Biotech	Spain	https://www.biorizon.es/en/