Emerging technologies of algae-based wastewater remediation for bio-fertilizer production: A promising pathway to the sustainable agriculture

Yao Zou [1], Qingqing Zeng [2], Huankai Li [2], Hui Liu [2,3]*, Qian Lu [4]*

[1] Guangdong Society of Environmental Sciences, Guangzhou 510045, China

[2] School of Resources and Environment, Zhongkai University of Agriculture and Engineering, Guangzhou, 510225, China

[3] Innovative Institute of Animal Healthy Breeding, Department of Environmental Science and Engineering, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, China

[4] School of Resources, Environmental & Chemical Engineering, Nanchang University, Nanchang 330031, China

* Correspondence: Hui Liu (liuhui@zhku.edu.cn); Qian Lu (luqian@ncu.edu.cn)

Abstract

Soil degradation, overuse of chemical fertilizer, and biodiversity loss are serious problems challenging the sustainable development of modern agriculture. In recent years, owing to the advantages of algae biotechnology in nutrients recovery and soil improvement, the integration of algae-based wastewater remediation and algal bio-fertilizer production is emerging into the limelight. In this work, we emphasize on the progresses achieved in the fields of biomass production by algae cultivation in wastewater and application of algal bio-fertilizers. Particularly, three types of algal bio-fertilizers, including slow-release bio-fertilizer, nitrogen-fixing cyanobacteria, and liquid bio-fertilizer, widely evaluated and utilized in agriculture are introduced.

To prevent the overly optimistic prediction of algal bio-fertilizer in a real-world application,

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jctb.6602

we point out the challenges and technical defects, such as presence of new pollution factors in wastewater, high cost of algal biomass, contamination of algal biomass in wastewater, low productivity of algae and valuable components, high water consumption, and potential threats of cyanobacteria to environment, that prevent the concept of integrating algae-based wastewater remediation and algal bio-fertilizer production from becoming a reality. Based on our knowledge and experience, potential solutions to the aforementioned problems are discussed in depth. It is expected that the emerging technologies of algae-based wastewater remediation for bio-fertilizer production will bring human beings into a new era of environmentally-friendly, high-yield, and resource-recycling agriculture.

Keywords: *Algae*; *Cyanobacteria*; *Bio-fertilizer*; *Wastewater remediation*; *Nutrients recovery*

Table of content

1. Introduction

The development of eco-friendly agriculture, in which the environmental pollutions and ecological disasters are controlled effectively, is a consensus of more and more people in the world [1]. However, the environmental and ecological problems, including the decrease of soil fertility, biodiversity loss, and contamination of underground water, are caused by the overuse of chemical fertilizers [2, 3]. As a category of organic fertilizer made from eukaryotic microalgae or prokaryotic cyanobacteria, algal fertilizer, which can capture carbon dioxide (CO_2), fix nitrogen, and improve soil fertility, is emerging into limelight [4, 5]. In addition, since algae are able to recover nutrients from wastewater, the algae cultivation could also be employed for wastewater remediation [6, 7]. Therefore, a novel concept of integrating wastewater-based algae culture and exploitation of biomass as bio-fertilizer was proposed and studied as an emerging technology for the eco-friendly agriculture [8, 9].

Current studies in this field cover algae-based wastewater remediation, bio-fertilizer processing, soil ecosystem protection, plants or crops growth, and food safety chain [10-12]. For example, Khan et al. (2019) proposed the concept of integrating wastewater-based algae culture and exploitation of biomass as bio-fertilizer. Three algal species, including *Chlorella minutissima*, *Scendesmus* sp., and *Nostoc muscorum*, were grown in sewage wastewater for nutrients removal and biomass synthesis and value-added biomass was harvested to produce bio-fertilizer [13]. The study of Khan et al. (2019) confirmed the practical feasibility of growing algae in wastewater to produce value-added bio-fertilizer. In addition to the research of Khan et al. (2019), many other studies explored the effects of algal bio-fertilizer on the microbial community in soil ecosystem and the contributions of algal bio-fertilizer to nitrogen fixation, providing a deep understanding of the positive roles of algal bio-fertilizer in eco-friendly agriculture [14, 15].

The algae-based wastewater remediation for bio-fertilizer production shows great advantages: (1) Wastewater could be obtained at very low cost, reducing the selling prices of algal bio-fertilizer; (2) The algae-based wastewater remediation could convert waste nutrients to value-added biomass, creating economic benefits [16]; (3) With the nutrients recovery by algae growth, potential pollution of the wastewater can be dramatically reduced [17]; (4) Harvested biomass can be exploited as algal bio-fertilizer for the eco-friendly agriculture. Owing to the great advantages in the economic and environmental aspects, the production and application of algal bio-fertilizer is becoming an emerging technology attracting attentions of researchers in both academia and industry.

Up to now, however, this emerging technology has not been widely used in agriculture. To our knowledge, some problems, such as high cost of algal bio-fertilizer, contamination of algal biomass, low productivity of algae and valuable components, water consumption and water loss, and potential threats of cyanobacteria to environment, are challenging the wide application of algal bio-fertilizer in agriculture worldwide [18, 19]. In the foreseeable future, to promote the use of algal bio-fertilizer in agriculture, aforementioned problems should be fully addressed.

In this paper, the application of algae biotechnology for wastewater remediation and bio-fertilizer production is reviewed and some emerging breakthroughs in this field are introduced. We would like to highly commend researchers on their meaningful studies related with algal bio-fertilizers, at the same time, discuss the problems challenging the use of algal bio-fertilizers in agricultural practice. By the end of this work, the prospects of the production and application of algal bio-fertilizers are discussed.

2. Technologies for the production and application of algal bio-fertilizer

Current studies associated with the algal bio-fertilizer can be classified into two important categories. Firstly, a portion of studies explored the algae-based nutrients recovery from wastewater for bio-fertilizer production. This novel technology is regarded as a feasible way to reduce the total cost of algal bio-fertilizer and alleviate the environmental pollution caused by wastewater discharge. Particularly, the conversion of waste resources to value-added bio-fertilizer will promote the development of circular economy in the agricultural industry. Secondly, a number of algal bio-fertilizer products were designed and evaluated in the agricultural practices. Up to now, three types of algal bio-fertilizer, including liquid bio-fertilizer, slow-release bio-fertilizer, and nitrogen-fixing cyanobacteria, made of algal biomass or living algal cells have been developed.

2.1. Algae-based wastewater remediation

To produce affordable bio-fertilizer, wastewater enriched with nutrients can be employed for algae cultivation. Compared with the artificial medium, wastewater could be obtained at low cost, thus reducing the total cost of algal biomass. Hence, the algae cultivation could be integrated with wastewater remediation for the biomass production. In this model, algae serve as an “intermediate carrier” to transport the wastewater nutrients to agricultural soil, bringing environmental benefit and economic benefit. Generally, nutrients removal and biomass composition are two important concerns in the algae-based wastewater remediation for bio-fertilizer production.

Nutrients removal

High removal efficiency of nutrient is expected in the algae-based wastewater remediation to ensure that the effluent can meet the requirements of current regulations. Up to now, it has been proven that a variety of agriculture-related wastewater, such as animal manure, food processing effluent, aquaculture wastewater, can be treated by the algae [7, 20, 21]. As shown in Table 1, algae including microalgae and cyanobacteria, could effectively remove a portion of nutrients in wastewater, and at the same time, produce biomass. In addition, compared with municipal wastewater and industrial effluent, agriculture-related wastewater contain no or much fewer toxic compounds and are more likely to be obtained in the rural areas. Therefore, the agriculture-related wastewater can be a good medium to cultivate algae for bio-fertilizer production.

However, it is noteworthy that in some cases the removal efficiency of nutrient in wastewater is low (Table 1). As a result, the discharge of wastewater after algae cultivation will not only cause environmental pollution, but also waste the nutrients in wastewater. Previous studies discovered that the low removal efficiency of nutrient is partly attributed to the unbalanced nutrients profile and the suspended organics in wastewater. To overcome this problem, a couple of novel solutions have been proposed and applied in practice. Firstly, wastewater from different sources could be mixed to balance the nutrients profile. For example, Leite et al. (2019) mixed municipal effluent and piggery wastewater to increase the nutrient concentration for microalgae cultivation.[22] This strategy could effectively improve the biomass yield and promote the removal of carbon and phosphorus (Table 1). Secondly, chemical oxidation can be employed to pretreat the wastewater, converting suspended organics to dissolved nutrients for algae growth. The chemical oxidation methods applied in the wastewater pretreatment process include the Fenton-iron oxidation, hypochlorite oxidation and so forth [7, 23]. Thirdly, the co-culture of algae with bacteria or yeast in wastewater could also be employed to promote the nutrients recovery [24]. On one hand, bacteria or yeast could degrade the suspended organics to low-molecular-weight organics, which are more likely to be assimilated by algal cells. On the other hand, algal photosynthesis could provide oxygen to bacteria or yeast performing heterotrophic metabolisms. Such a symbiotic relationship could not only promote the nutrients recovery, but also improve the biomass yield.

Biomass composition

The fertilization effect of algal bio-fertilizer is partly attributed to the biomass composition. To our knowledge, owing to the importance of nitrogen element to plants growth, nitrogen-rich biomass is regarded as a good feedstock for algal bio-fertilizer production. Nitrogen in wastewater is important to the nitrogen accumulation and protein synthesis in algal biomass, but ammonia toxicity may limit the algae growth or even cause the failure of algae cultivation. For example, in the study of Lu et al. (2018), when the concentration of ammonia reached 28.03 mM, the growth of *Chlorella* sp. in aqueous phase was inhibited [25].

To alleviate the ammonia toxicity in wastewater, previous studies employed a variety of pretreatment methods.[26, 27] For example, the pretreatment by nitrification and ammonia stripping could reduce the concentration of ammonia in wastewater via nitrifying bacteria activity and air bubbling, respectively, thus creating a favorable environment for algae growth. In addition, Lu et al. (2019) reported the feasibility of using zeolite to absorb ammonia at the initial stage while release ammonia into wastewater at the later stage for the growth of *Spirulina* sp. [26] The addition of zeolite in wastewater could not only alleviate the ammonia toxicity, but also mitigate the nitrogen deficiency at the later stage of algae cultivation. Owing to the continuous supply of nitrogen by zeolite, algae with high protein content (69.8% of total dry weight) and high biomass yield (4.31 g/L) were obtained [26].

2.2. Application of algal bio-fertilizer

Current studies cover both fundamental research and applied research, fully documenting the positive effects of algal bio-fertilizers on plant growth, crop yield, soil microorganisms, nutritional value of fruits, and seeds germination [10, 11, 28]. Driven by the ecological and economic benefits, researchers from academia and industry developed three major models to utilize the algal bio-fertilizers in agriculture (Figure 1). Firstly, algal biomass, which could be degraded by the soil microorganisms, serves as slow-release fertilizer to continuously provide nutrients to plants [29, 30]. Secondly, living algal cells could be added into soil to regulate the microbial community, improve soil fertility, and control soil moisture [15, 31]. Thirdly, algae extract containing amino acids and minerals can be exploited as liquid bio-fertilizer spread on the surface of plant leaves [32]. Although slow-release bio-fertilizer, nitrogen-fixing cyanobacteria, and liquid bio-fertilizer, are different in the production processes and action mechanisms, all of them can have very positive effects on plant growth and soil quality (Table 2).

Slow-release bio-fertilizer

According to previous studies, the algae that can be cultivated as the feedstock of slow-release bio-fertilizer include *Nannochloropsis* sp., *Chlorella* sp. and *Spirulina* sp., and so on [30, 33]. Most of the algae exploited for slow-release bio-fertilizer have high content of protein, of which the degradation in soil can release a large amount of nitrogen [30]. The processes of biomass degradation and nutrients release are catalyzed by the microorganisms in soil. It was reported that *Proteobacteria* (35-42%), *Acidobacteria* (12-15%), and *Bacteroidetes* (8-10%), became the most abundant microbial species in the cucumber rhizosphere when algal bio-fertilizer was added in the soil [34]. The biomass degradation catalyzed by the microbial activities is a slow process, increasing the effective duration of algal bio-fertilizer. Besides, algal biomass, microorganisms, and inorganic particles can form an adherent network in soil, reducing the nutrients loss of fertilizer in the process of irrigation [35, 36]. Therefore, the slow-release algal fertilizer shows greater advantages over traditional chemical fertilizer.

Normally, the production process of slow-release algal fertilizer involves the algae cultivation, biomass dehydration, and biomass pasteurization or pulverization [33]. It is noteworthy that the dehydration process is to lower the moisture content in algal biomass and prevent the excessive deterioration of bio-fertilizer during storage. In some cases, this process, together with algae cultivation and biomass harvesting, might be highly energy-intensive, resulting in the high cost of algal bio-fertilizer. In the study of Lv et al. (2020), microalgae biomass was suspended in the tap water as bio-fertilizer to support the growth of cucumber without dehydration or pasteurization [34]. In this way, the energy consumption and total cost of the algal bio-fertilizer can be dramatically reduced. In addition, Rothlisberger-Lewis et al. (2016) used the lipid-extracted algae as the feedstock of bio-fertilizer, thus ensuring the integration of biodiesel production and bio-fertilizer production [37]. The aforementioned novel technologies can be regarded as important attempts to producing affordable algal bio-fertilizer.

The important roles of slow-release algal fertilizer in agriculture mainly include the support of plant growth and the improvement of soil quality. Firstly, the nutrients, such as nitrogen, phosphorus, organic carbon, and minerals, released from algal fertilizer can be assimilated by plants. Coppens et al. (2016) reported that in the growth of tomatoes, the addition of algal bio-fertilizer not only increased the leaf length and leaf fresh weight, but also promoted the accumulation of glucose, fructose, and carotenoids in tomato fruits [33]. The positive effects of algal bio-fertilizer on plant growth were also reported by many other studies (Table 2). Secondly, the nutrients released from algal fertilizer could improve the soil fertility. It was reported that algal bio-fertilizer increased the contents of available nitrogen, phosphorus, and potassium in soil, providing the plants with more sufficient nutrients [29]. Also, at the harvest stage, the contents of nitrogen, phosphorus, and potassium remained in the soil fertilized by algal biomass were higher than the contents of nutrients in other experimental groups. Hence, the use of algal bio-fertilizer could protect the soil quality in a long period of time.

Nitrogen-fixing cyanobacteria

The nitrogen-fixing cyanobacteria refer to a category of blue-green algae which could absorb nitrogen directly from air. To our knowledge, not all the blue-green algae have the ability of assimilating nitrogen in air and the nitrogen-fixing cyanobacteria mainly include the genera *Nostoc* and *Anabaena* [38]. The living cyanobacteria used as bio-fertilizer could not only provide nitrogen to agricultural crops continuously, but also improve the soil quality and protect the plants or crops [12, 39].

The use of nitrogen-fixing cyanobacteria in agriculture shows advantages in five aspects. Firstly, with the metabolisms of nitrogen-fixing cyanobacteria, nitrogen in atmosphere can be transported to soil to support the plants growth. It was reported that cyanobacteria fertilizer could add up to 20-30 kg nitrogen ha^{-1} to agricultural crops [12, 39]. Thus, the application amount of chemical nitrogen fertilizer in agriculture can be lowered. Secondly, cyanobacteria could release extracellular polymeric substances and plant growth-promoting (PGP) substance, which can improve the soil quality and support the plants growth, respectively [40]. Specific contributions of nitrogen-fixing cyanobacteria to soil quality improvement and plants growth are presented in Table 2. Thirdly, some cyanobacteria could lower the incidence rate of agricultural diseases. Previous studies reported that cyanobacteria could limit the growth or invasion of pathogenic microorganisms, such as *Fusarium wilt*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Alternaria porri* [41, 42]. Fourthly, cyanobacteria could support the formation of biological soil crust, which plays a key role in the prevention of land desertification. Tiwari et al. (2019) discovered that exopolysaccharides (EPSs) secreted by nitrogen-fixing cyanobacteria mainly contributed to the consolidated structure of soil crust, thus retaining the humidity in soil and improving the water availability [43]. Last but not the least, as algae can be regarded as a huge carbon sink, the living cyanobacteria in soil will partly compensate the carbon dioxide (CO_2) emission of agriculture [44]. Therefore, the wide application of nitrogen-fixing cyanobacteria can be a promising pathway to the sustainable agriculture.

In the practice, cyanobacteria can be applied in the forms of microbial inoculant and microbial biofilm [45]. Firstly, it is the most straightforward way to add cyanobacteria cells in soil as microbial inoculant. Previous studies discovered that cyanobacteria could improve the soil quality and promote the plants growth (Table 2). Nevertheless, in some cases, microbial inoculants may suffer from the problem of poor survival in soil and their PGP abilities are dependent upon their competence in this niche [45]. Secondly, the cyanobacteria biofilm can be prepared before the inoculation of microorganisms in soil. In a real-world application, to improve the performance of biofilm in soil protection, cyanobacteria can be mixed with other microorganisms in the preparation of biofilm [45].

Liquid bio-fertilizer

Liquid bio-fertilizers mainly refer to the algal extracts enriched with the nutrients essential to plants growth (Table 2). In the foliar application of algal extracts, nutrients could enter the plants or crops via leaf pores. Compared with the slow-release fertilizer, the liquid bio-fertilizer can be utilized by plants and crops in a more efficient way. Also, with the wide application of liquid bio-fertilizer in agriculture, the problems, such as fast growth of weeds after fertilization, nutrients uptake restricted by low temperature, and nutrients loss after irrigation, caused by the traditional fertilizers can be alleviated effectively. Hence, the use of liquid bio-fertilizer made from algal biomass is becoming an emerging trend in eco-friendly agriculture.

To produce the liquid bio-fertilizer, it is an essential step to break the cell wall of algae in a cost-saving and efficient way. Up to now, the methods that can be applied to break the cell wall include high pressure gases treatment, bead milling, ultrasound treatment, alkaline digestion, enzymatic degradation, and so on. (1) High pressure gases treatment, bead milling, and ultrasound treatment belong to the physical methods, which break the structure of algal cells by physical impacts or shocks [46]. However, it is noteworthy that some physical methods have high energy consumption and require special equipment. (2) Enzymatic degradation refers to the method of adding cellulase, mannanase, xylanase or pectinase to degrade certain compounds in cell wall [47, 48]. Compared with the physical methods and chemical methods, the enzymatic degradation can be conducted in a much milder environment, alleviating the damage of cell wall breakage to the value-added biomass compositions [47]. Some parameters, such as pH, incubation time, temperature, and enzyme concentration, should be optimized to improve the performance of enzymatic degradation in the breakage of cell wall. In some cases, to improve the treatment efficiency, aforementioned methods can be combined for the breakage of algal cell wall.

As shown in Table 2, liquid bio-fertilizer could effectively promote the growth of plants or crops. For example, Vijayakumar et al. (2019) discovered that the liquid bio-fertilizer provided amino acids and essential minerals to *Capsicum annum*, improving the root length, total dry weight, leaf area, and the number of branches and pods [11]. The positive effects of algal bio-fertilizers on the growth of other plants or crops, such as *Vigna radiata*, *Cucumis sativus*, and *Oryza sativa* were also reported [10, 49]. Therefore, the algal bio-fertilizer industry is of vital importance to the agricultural development.

3. Challenges and technical defects

Although previous studies optimized the cultivation conditions for microalgae-based wastewater remediation and explored the positive effects of algal bio-fertilizers on ecosystem and agriculture, there are a couple of problems challenging the emerging technology of algae-based wastewater remediation for bio-fertilizer production (Figure 2).

Presence of new pollution factors in wastewater

It has been widely documented that algae could remove the nutrients, such as nitrogen, phosphorus and organic carbon, in wastewater [7, 50]. However, this does not mean that algae culture is a perfect technology for wastewater remediation since new pollution factors may be introduced into the aqueous phase. Firstly, algae growth could increase the pH value of wastewater and the alkaline wastewater is not allowed to be directly discharged. In the study of Khan et al. (2019), the pH value of wastewater reached 9.32 after algae culture [13]. Cardoso et al. (2020) demonstrated that the growth of *Spirulina* sp., a category of cyanobacteria usually used as feedstock of bio-fertilizer, even increased the pH of culture medium to 10.60 [50]. Secondly, a number of prokaryotic cyanobacteria could release toxic components, causing the ecological disasters in water body [51]. For example, *Nostoc muscorum*, which was used in the study of Khan et al. (2019), could synthesize microcystins [52]. Thirdly, after wastewater treatment, a portion of living algae may be left in the aqueous phase since most techniques could not ensure 100% harvesting efficiency. With the discharge of wastewater, the living cyanobacteria will enter waters, causing algal bloom and disturbing ecological balance.

Owing to the new pollution factors brought by algae, particularly cyanobacteria, the roles of algae in wastewater remediation should be comprehensively assessed. In our view, these new pollution factors, particularly the cyanobacteria toxins and the alkalization, might be more threatening to environment than eutrophic wastewater.

High cost of algal biomass

High production cost is one of the serious problems hindering the industrial application of algal biomass. The process of algal bio-fertilizer production consists of algae strains screening, algae culture, algae harvesting, biomass dehydration, and fertilizer preparation, making the costs of biomass and algal bio-fertilizer remarkably high [53] It was reported that in current microalgae production systems, the biomass production cost ranges between 5 €/kg for open raceway pond and 50 €/kg for photo-bioreactor [54]. In the study of Acién et al. (2012), the total cost of algal biomass was reduced from 69 €/kg to 12.6 €/kg by scaling up the production capacity [53]. If the nitrogen content in dry biomass was set as 5.87%, the unit cost of nitrogen element will be over 214.65 €/kg. As a result, the algal bio-fertilizer, showing no advantages over chemical fertilizer in the aspect of production cost, would not be affordable to farmers [18, 55]. Owing to the low profitability of traditional agriculture in the developing countries, it is not the best choice for farmers to use the algal bio-fertilizer to replace chemical fertilizer in the large-scale agricultural production.

Contamination of algal biomass

Some wastewater containing toxic pollutants, particularly heavy metals, could contaminate the algal biomass, resulting in the accumulation of toxic pollutants in soil and crops [56]. In addition, algae, of which the cell surface is enriched with functional groups, such as hydroxyl and carboxyl, are negatively charged, performing well in the adsorption of heavy metals [57, 58] Hence, algae culture is a process not only assimilating nutrients from culture media, but also adsorbing and accumulating heavy metals [57]. Heavy metals could enter algal cells either by means of active transport or by endocytosis through chelating proteins [59]. According to the report of Kumar et al. (2015), the maximum uptake rates of Cd, Cr, Cu, Hg, and Pb by living algal cells could reach 105 mg/g, 304 mg/g, 576 mg/g, 15 mg/g and 188 mg/g, respectively [60]. As a result, the algae culture in some wastewater might become a process of adsorbing and accumulating heavy metals. With the degradation of bio-fertilizer in soil, the heavy metals concentrated in algal biomass would be released, resulting in the contamination of farmland and plants.

Continuous use of wastewater for irrigation could result in the accumulation of heavy metals in soil and eventually lead to increased uptake of heavy metals by crops and plants [61]. Hence, the soil irrigation by wastewater, which can cause serious ecological disasters and environmental pollutions, is strictly prohibited in many countries. In our view, if heavy metals in wastewater are not treated properly, the use of algal bio-fertilizer for soil fertilization will be another form of irrigating farmland by using wastewater since algal bio-fertilizer could transport a portion of toxic pollutants into soil.

Low productivity of algae and valuable components

To meet the requirement of plant growth, nitrogen and phosphorus should be provided continuously through fertilization. However, the productivity of nitrogen and phosphorus in algae culture are too low to support the wide application of algal bio-fertilizer. In the study of Khan et al. (2019), the period of phytoremediation and algae culture were set as 25-day, but the biomass yields of algae were only 0.14-0.45 g/L [13]. If the maximum contents of nitrogen and phosphorus in algal biomass were set as 5.87% and 0.95%, respectively [13], the productivity of nitrogen and phosphorus in algae grown in sewage wastewater were only 0.329-1.057 g/m^3/day and 0.053-0.171 g/m^3/day. If these data are applicable in industry, to produce bio-fertilizer with 1 ton nitrogen per day, 9.464×10^5-3.042×10^6 m^3 sewage wastewater and 1.89-6.08 km^2 areas are needed (Table 3).

During the year of 2019, 3.696×10^7 ton nitrogen fertilizer was produced in China to meet the demand of agriculture. In this case, to replace the chemical nitrogen fertilizer with algal bio-fertilizer, 1.914×10^5-6.157×10^5 km^2 areas, accounting for 2.00-6.41% of all the land of China (9.6×10^6 km^2), will be needed for algae culture. In a real-world application, it is not feasible to use such a huge land area for the production algal bio-fertilizer. Therefore, owing to the low productivity of algae and valuable components, the total production capacity of algal bio-fertilizer will be very limited, further hindering the wide use of bio-fertilizer in agriculture.

Water consumption and water loss

Algae production is a water-consuming process, which requires the input of a large volume of water and generates wastewater containing residual nutrients or other polluting agents [62]. Normally, biomass yields of microalgae and cyanobacteria in autotrophic model could reach

0.5-4.0 kg/m^3, suggesting that around 1 ton water would be consumed to produce 0.5-4.0 kg algal biomass. In addition, since open pond is usually employed to produce algae at a large scale, water loss caused by evaporation should be considered. It was reported that the evaporation rates of lakes in tropical zone and temperature zone could reach 5.1 mm/day and 3.1 mm/day, respectively [63, 64]. In other words, during a 10-day culture period, about 3.1-5.1 m^3 water will be lost by evaporation in a 100 m^2 open pond for algae production. Thus, the high water consumption and water loss of algae culture may cause serious environmental problems or ecological disasters if algal bio-fertilizers are widely produced and used in the arid or semi-arid countries.

Potential threats of cyanobacteria to environment

Living cyanobacteria are added into soil as bio-fertilizer to fix atmospheric nitrogen and convert it into an available form at no cost for plant growth, but the potential threats, such as cyanobacteria toxin and biological invasion, are neglected [65-67]. Firstly, some cyanobacteria could release toxins, such as microcystins, nodularins, aplysiatoxins, cylindrospermopsin, saxitoxins, anatoxin, and lyngbyatoxin, which might contaminate the soil or even the underground water [68]. It was even observed that microcystins were accumulated in salad lettuce and clover, threatening the safety of food chain [69, 70]. Secondly, living cyanobacteria used as bio-fertilizers may migrate to the water body near farmland, resulting in algal bloom and biological invasion [65]. This problem is remarkably threatening to the farm ecosystem of modern agriculture which usually integrates crops planting and fish culturing.

In addition to the aforementioned problems, some other unfavorable factors, such as the residual nutrients in wastewater after algae cultivation, inconvenience in the transportation and storage of liquid bio-fertilizer, uncontrolled degradation rate of algal biomass used as slow-release bio-fertilizer, and low consumer-acceptance of bio-fertilizers, are hindering the application of algal bio-fertilizer in agriculture worldwide.

4. Potential solutions and prospects

The presentation of problems challenging the production and application of algal bio-fertilizer is not to deny its importance and promising prospects. According to our judgment, some technical improvement, novel concept, and policy support can be good solutions to the aforementioned problems and support the wide use of algal bio-fertilizer.

Nutrients recovery from wastewater with a high level of safety

Wastewater-based algae growth is a promising way to reduce the biomass production cost, but only the wastewater with a high level of safety should be used to obtain the pollutants-free biomass. To our knowledge, food processing effluent, slaughterhouse effluent, and straw fermentation wastewater without toxic components could be regarded as the wastewater with a high level of safety [71, 72]. In addition, the wastewater from food or feed industries can be easily obtained at no cost in rural areas. Thus, the use of "highly-safe" wastewater from food or feed industries to grow algae and produce bio-fertilizer will realize the resource reutilization in rural areas and support the development of circular-economy.

Desorption of heavy metals on algal cells

Heavy metals could be combined with algal cells in the forms of extracellular adsorption and intracellular chelation. Normally, heavy metals adsorbed on algal cells are more likely to be removed by desorption treatment, thus reducing the total amount of heavy metals in algal biomass. Previous studies have discovered that the addition of chelating agents and the pH adjustment are effective methods to promote desorption of heavy metals and studied the desorption kinetics [73]. Particularly, the desorption treatment methods are affordable and will not dramatically increase the total cost of algal bio-fertilizer. Therefore, it may be a feasible way to prevent the soil contamination caused by the heavy metals in bio-fertilizers by conducting appropriate desorption treatment.

Integrated culture of algae and bacteria for water-quality control

As documented by previous study, residual nutrients and increased pH value are the main water-quality problems in the wastewater after algae growth [74]. The integrated culture of algae and bacteria can be a potential solution to the water-quality problems. Firstly, bacteria could degrade the suspended organic solids in wastewater to dissolved nutrients available for algae growth. At the same time, oxygen produced by photosynthetic algae could support the bacterial growth and metabolisms. The algal-bacterial cooperation has been proven to be an effective way for nutrients recovery in wastewater [24]. Secondly, some bacteria could convert organics in wastewater to volatile fatty acids, such as acetic acid, propionic acid, and butyric acid, resulting in the decrease of pH value of wastewater [75]. Thus, the pH increase caused by algae growth will be alleviated by the bacterial activities.

Water loss control in microalgae cultivation

To overcome the problems of water shortage in arid or semi-arid countries, immobilized culture method and closed photo-bioreactor are considered as possible ways to produce algal biomass with low water consumption. Immobilized culture refers to the method of growing microalgae on a substrate or film [76]. In some cases, the water can be sprayed on the surface of film, thus reducing the water loss caused by evaporation to a lower level. In addition, the closed photo-bioreactor, which has much smaller area directly exposed to air, may have lower water evaporation than open raceway pond [77]. In a real-world application, more efforts should be devoted into the assessment of water loss and water input of these methods under specific conditions.

Utilization of non-toxic microorganisms

Non-toxic microorganisms can be utilized to prevent the potential threats of cyanobacteria toxins to ecosystem. To our knowledge, some microorganisms, including *Rhodospirillum rubrum*, *Bacillus pumilus*, and *Bacillus subtilis* perform well in nitrogen fixation and have no toxic effects on plants and ecosystem [78-80]. Besides, *Bacillus* sp. which are widely spread in atmosphere, farmland, and water, will not cause biological invasion even if they enter into the soil and water via wastewater or irrigation water. Previous studies widely documented the use of these non-toxic microorganisms in farmland management and proved their positive effects on plant growth and environmental protection [78, 81]. Some of these non-toxic microorganisms are regarded as probiotics, of which the accumulation in food chain will not threaten the animals' or humans' health. In our view, it can be a promising way to alleviate the threats of cyanobacteria toxins to ecosystem by employing non-toxic microorganisms for nitrogen fixation in farmland.

However, one of the challenges to the use of *Bacillus* sp. as bio-fertilizer is that the harvesting cost of bacterial biomass is much higher than that of filamentous cyanobacteria. Normally, filamentous cyanobacteria could be efficiently harvested via simple sedimentation or filtration while the thickening process of bacteria is time-consuming and even energy-intensive [82]. Hence, to promote the use of beneficial bacteria, such as *Bacillus* sp. as bio-fertilizer for nitrogen fixation in soil, the biomass thickening techniques should be improved.

Policy support, government subsidy and international cooperation

The use of algal bio-fertilizer could effectively minimize greenhouse effect since the algae culture is a carbon-absorbing process while the production of chemical fertilizer releases a huge amount of CO_2 into atmosphere [83]. Therefore, the benefits brought by algal bio-fertilizer industry to society and natural environment are numerous. In our view, it is necessary for the governments to include the algal bio-fertilizer industries into carbon trading market and provide subsidy to reward their contributions to environmental protection. With the policy support and government subsidy, the financial burden of algal bio-fertilizer industries will be relieved and the development of sustainable agriculture will be promoted.

The international cooperation is also important to the wide use of algal bio-fertilizer. At present, the technologies for the production and application of algal bio-fertilizer are very novel and challenged by some potential problems or risks. Hence, the international cooperation is highly needed to promote the information exchange and support the sustainable development of algal bio-fertilizer industry.

5. Conclusion

In conclusion, greater awareness regarding the benefits of algae-based wastewater remediation for bio-fertilizer production is increasing and there is huge market potential for algal bio-fertilizer industry. In our view, the strategies of converting wasted resources to algal bio-fertilizer, desorbing heavy metals on algal cells, controlling water loss in algae cultivation, employing non-toxic microorganisms, and proving policy support and government subsidy can partly solve current problems of algal bio-fertilizer and support the sustainable development of agriculture. It is expected that the technical advancement and policy support will bring an epoch-making breakthrough to algal bio-fertilizer industry, bringing mankind into a new era of environmentally-friendly, high-yield, and resource-recycling agriculture.

Acknowledgements

This work was funded by Natural Science Foundation of Guangdong Province (Nos. 2018A030313425, 2018A030313696), Guangzhou Municipal Science and Technology Project (Nos. 201807010056, 201907010056), Guangdong Provincial Special Fund For Modern Agriculture Industry Technology Innovation Teams (2019KJ141) and Zhongkai Foundation (KA200540504).

Credit Author Statement

Yao Zou: Writing - Original Draft; Revision

Qingqing Zeng: Revision

Huankai Li: Writing - Original Draft

Hui Liu: Conceptualization; Revision

Qian Lu: Conceptualization

References

1. Massah J, Azadegan BJ, Effect of chemical fertilizers on soil compaction and degradation. *Agricultural Mechanization in Asia, Africa & Latin America* **47**: 44-50 (2016).
2. Bai X, Wang Y, Huo X, Salim R, Bloch H and Zhang H, Assessing fertilizer use efficiency and its determinants for apple production in China. *Ecological Indicators* **104**: 268-278 (2019).
3. Srivastav AL, Chapter 6 - Chemical fertilizers and pesticides: role in groundwater contamination, in Agrochemicals Detection, Treatment and Remediation, ed by Prasad MNV. Butterworth-Heinemann, pp. 143-159 (2020).
4. Chojnacka K, Moustakas K and Witek-Krowiak A, Bio-based fertilizers: A practical approach towards circular economy. *Bioresource Technology* **295**: 122223 (2020).
5. Singh JS, Kumar A and Singh M, Cyanobacteria: A sustainable and commercial bio-resource in production of bio-fertilizer and bio-fuel from waste waters. *Environmental and Sustainability Indicators* **3-4**: 100008 (2019).
6. Papadopoulos KP, Economou CN, Tekerlekopoulou AG and Vayenas DV, Two-step

treatment of brewery wastewater using electrocoagulation and cyanobacteria-based cultivation. *Journal of Environmental Management* **265**: 110543 (2020).

7. Hu X, Meneses YE and Hassan AA, Integration of sodium hypochlorite pretreatment with co-immobilized microalgae/bacteria treatment of meat processing wastewater. *Bioresource technology* **304**: 122953 (2020).

8. Solovchenko A, Verschoor AM, Jablonowski ND and Nedbal L, Phosphorus from wastewater to crops: An alternative path involving microalgae. *Biotechnology Advances* **34**: 550-564 (2016).

9. Renuka N, Guldhe A, Prasanna R, Singh P and Bux F, Microalgae as multi-functional options in modern agriculture: current trends, prospects and challenges. *Biotechnology Advances* **36**: 1255-1273 (2018).

10. Deepika P, MubarakAli DJB and Biotechnology A, Production and assessment of microalgal liquid fertilizer for the enhanced growth of four crop plants. *Biocatalysis and Agricultural Biotechnology* **28**: 101701 (2020).

11. Vijayakumar S, Durgadevi S, Arulmozhi P, Rajalakshmi S, Gopalakrishnan T and Parameswari N, Effect of seaweed liquid fertilizer on yield and quality of *Capsicum annum* L. *Acta Ecologica Sinica* **39**: 406-410 (2019).

12. Singh JS, Kumar A, Singh M, Cyanobacteria: a sustainable and commercial bio-resource in production of bio-fertilizer and bio-fuel from waste waters. *Environmental and Sustainability Indicators* **3**: 100008 (2019).

13. Khan SA, Sharma GK, Malla FA, Kumar A, Rashmi and Gupta N, Microalgae based biofertilizers: A biorefinery approach to phycoremediate wastewater and harvest biodiesel and manure. *Journal of Cleaner Production* **211**: 1412-1419 (2019).

14. Peng X and Bruns MA, Development of a nitrogen-fixing cyanobacterial consortium for surface stabilization of agricultural soils. *Journal of Applied Phycology* **31**: 1047-1056 (2019).

15. Acea MJ, Diz N and Prieto-Fernandez A, Microbial populations in heated soils inoculated with cyanobacteria. *Biology and Fertility of Soils* **33**: 118-125 (2001).

16. Kadir WNA, Lam MK, Uemura Y, Lim JW and Lee KT, Harvesting and pre-treatment of microalgae cultivated in wastewater for biodiesel production: A review. *Energy Conversion and Management* **171**: 1416-1429 (2018).

17. Gupta S, Pawar SB and Pandey RA, Current practices and challenges in using microalgae for treatment of nutrient rich wastewater from agro-based industries. *Science of The Total Environment* **687**: 1107-1126 (2019).

18. Vandamme D, Foubert I and Muylaert K, Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. *Trends in Biotechnology* **31**: 233-239 (2013).

19. Leong YK and Chang J-S, Bioremediation of heavy metals using microalgae: Recent advances and mechanisms. *Bioresource technology* **303**: 122886 (2020).

20. Luo L, Ren H, Pei X, Xie G, Xing D, Dai Y, Ren N and Liu B, Simultaneous nutrition removal and high-efficiency biomass and lipid accumulation by microalgae using anaerobic digested effluent from cattle manure combined with municipal wastewater. *Biotechnology for Biofuels* **12**: 218 (2019).

21. Guerrero-Cabrera L, Rueda JA, Garcia-Lozano H and Karin Navarro A, Cultivation of *Monoraphidium* sp., *Chlorella* sp and *Scenedesmus* sp algae in Batch culture using *Nile tilapia* effluent. *Bioresource technology* **161**: 455-460 (2014).

22. Leite LdS, Hoffmann MT and Daniel LA, Microalgae cultivation for municipal and piggery wastewater treatment in Brazil. *Journal of Water Process Engineering* **31**: 100821 (2019).

23. Zhang F, Yue Q, Gao Y, Gao B, Xu X, Ren Z and Jin Y, Application for oxytetracycline wastewater pretreatment by Fenton iron mud based cathodic-anodic-electrolysis ceramic granular fillers. *Chemosphere* **182**: 483-490

(2017).

24. Liu H, Lu Q, Wang Q, Liu W, Wei Q, Ren H, Ming C, Min M, Chen P and Ruan R, Isolation of a bacterial strain, *Acinetobacter* sp from centrate wastewater and study of its cooperation with algae in nutrients removal. *Bioresource technology* **235**: 59-69 (2017).

25. Lu Q, Chen P, Addy M, Zhang R, Deng X, Ma Y, Cheng Y, Hussain F, Chen C, Liu Y and Ruan R, Carbon-dependent alleviation of ammonia toxicity for algae cultivation and associated mechanisms exploration. *Bioresource technology* **249**: 99-107 (2018).

26. Lu Q, Han P, Chen F, Liu T, Li J, Leng L, Li J and Zhou W, A novel approach of using zeolite for ammonium toxicity mitigation and value-added Spirulina cultivation in wastewater. *Bioresource technology* **280**: 127-135 (2019).

27. Li J, Wang L, Lu Q and Zhou W, Toxicity alleviation for microalgae cultivation by cationic starch addition and ammonia stripping and study on the cost assessment. *Rsc Advances* **9**: 38235-38245 (2019).

28. Renuka N, Guldhe A, Prasanna R, Singh P and Bux FJBa, Microalgae as multi-functional options in modern agriculture: current trends, prospects and challenges. **36**: 1255-1273 (2018).

29. Saadaoui I, Sedky R, Rasheed R, Bounnit T, Almahmoud A, Elshekh A, Dalgamouni T, al Jmal K, Das P and Al Jabri H, Assessment of the algae-based biofertilizer influence on date palm (*Phoenix dactylifera* L.) cultivation. *Journal of Applied Phycology* **31**: 457-463 (2019).

30. Dineshkumar R, Kumaravel R, Gopalsamy J, Sikder MNA and Sampathkumar P, Microalgae as Bio-fertilizers for Rice Growth and Seed Yield Productivity. *Waste and Biomass Valorization* **9**: 793-800 (2018).

31. Sepehr A, Hassanzadeh M and Rodriguez-Caballero E, The protective role of cyanobacteria on soil stability in two Aridisols in northeastern Iran. *Geoderma*

Regional **16**: e00201 (2019).

32. Deepika P and Mubarakali D, Production and assessment of microalgal liquid fertilizer for the enhanced growth of four crop plants. *Biocatalysis and Agricultural Biotechnology* **28**: 101701 (2020).

33. Coppens J, Grunert O, Van den Hende S, Vanhoutte I, Boon N, Haesaert G and De Gelder L, The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. *Journal of Applied Phycology* **28**: 2367-2377 (2016).

34. Lv J, Liu S, Feng J, Liu Q, Guo J, Wang L, Jiao X and Xie S, Effects of microalgal biomass as biofertilizer on the growth of cucumber and microbial communities in the cucumber rhizosphere. *Turkish Journal of Botany* **44**: 167 (2020).

35. Kheirfam H, Sadeghi SH and Darki BZ, Soil conservation in an abandoned agricultural rain-fed land through inoculation of cyanobacteria. *Catena* **187**: 104341 (2020).

36. Colica G, Li H, Rossi F, Li D, Liu Y and De Philippis R, Microbial secreted exopolysaccharides affect the hydrological behavior of induced biological soil crusts in desert sandy soils. *Soil Biology & Biochemistry* **68**: 62-70 (2014).

37. Rothlisberger-Lewis KL, Foster JL and Hons FM, Soil carbon and nitrogen dynamics as affected by lipid-extracted algae application. *Geoderma* **262**: 140-146 (2016).

38. Gupta V, Ratha SK, Sood A, Chaudhary V and Prasanna R, New insights into the biodiversity and applications of cyanobacteria (blue-green algae)-Prospects and challenges. *Algal Research-Biomass Biofuels and Bioproducts* **2**: 79-97 (2013).

39. Karthikeyan N, Prasanna R, Sood A, Jaiswal P, Nayak S and Kaushik BJFM, Physiological characterization and electron microscopic investigation of cyanobacteria associated with wheat rhizosphere. *Folia Microbiol* **54**: 43-51 (2009).

40. Li H, Zhao Q and Huang H, Current states and challenges of salt-affected soil

remediation by cyanobacteria. *Science of the Total Environment* **669**: 258-272 (2019).

41. Roberti R, Galletti S, Burzi PL, Righini H, Cetrullo S and Perez C, Induction of defence responses in zucchini (*Cucurbita pepo*) by *Anabaena* sp. water extract. *Biological Control* **82**: 61-68 (2015).

42. Prasanna R, Babu S, Bidyarani N, Kumar A, Triveni S, Monga D, Mukherjee AK, Kranthi S, Gokte-Narkhedkar N, Adak A, Yadav K, Nain L and Saxena AK, Prospecting cyanobacteria-fortified composts as plant growth promoting and biocontrol agents in cotton. *Experimental Agriculture* **51**: 42-65 (2015).

43. Tiwari ON, Bhunia B, Mondal A, Gopikrishna K and Indrama T, System metabolic engineering of exopolysaccharide-producing cyanobacteria in soil rehabilitation by inducing the formation of biological soil crusts: A review. *Journal of Cleaner Production* **211**: 70-82 (2019).

44. Wang R, Peng B and Huang K, The research progress of CO_2 sequestration by algal bio-fertilizer in China. *Journal of CO_2 Utilization* **11**: 67-70 (2015).

45. Swarnalakshmi K, Prasanna R, Kumar A, Pattnaik S, Chakravarty K, Shivay YS, Singh R and Saxena AK, Evaluating the influence of novel cyanobacterial biofilmed biofertilizers on soil fertility and plant nutrition in wheat. *European Journal of Soil Biology* **55**: 107-116 (2013).

46. Yong TC, Chiu P-H, Chen C-H, Hung C-H and Chen C-NN, Disruption of thin- and thick-wall microalgae using high pressure gases: Effects of gas species, pressure and treatment duration on the extraction of proteins and carotenoids. *Journal of bioscience and bioengineering* **129**: 502-507 (2020).

47. Zhang Y, Kong X, Wang Z, Sun Y, Zhu S, Li L and Lv P, Optimization of enzymatic hydrolysis for effective lipid extraction from microalgae *Scenedesmus* sp. *Renewable Energy* **125**: 1049-1057 (2018).

48. Maffei G, Bracciale MP, Broggi A, Zuorro A, Santarelli ML and Lavecchia R, Effect

of an enzymatic treatment with cellulase and mannanase on the structural properties of *Nannochloropsis* microalgae. *Bioresource technology* **249**: 592-598 (2018).

49. Yao Y, Zhang M, Tian Y, Zhao M, Zeng K, Zhang B, Zhao M and Yin B, Azolla biofertilizer for improving low nitrogen use efficiency in an intensive rice cropping system. *Field Crops Research* **216**: 158-164 (2018).

50. Cardoso LG, Duarte JH, Andrade BB, Franca Lemos PV, Vieira Costa JA, Druzian JI and Chinalia FA, *Spirulina* sp. LEB 18 cultivation in outdoor pilot scale using aquaculture wastewater: High biomass, carotenoid, lipid and carbohydrate production. *Aquaculture* **525**: 735272 (2020).

51. Dittmann E, Fewer DP and Neilan BA, Cyanobacterial toxins: biosynthetic routes and evolutionary roots. *Fems Microbiology Reviews* **37**: 23-43 (2013).

52. Oudra B, Andaloussi MD-E and Vasconcelos VM, Identification and quantification of microcystins from a *Nostoc muscorum* bloom occurring in Ouka meden River (High-Atlas mountains of Marrakech, Morocco). *Environmental Monitoring and Assessment* **149**: 437-444 (2009).

53. Acien FG, Fernandez JM, Magan JJ and Molina E, Production cost of a real microalgae production plant and strategies to reduce it. *Biotechnology Advances* **30**: 1344-1353 (2012).

54. Fernández FGA, José María Fernández S and Grima EM, Costs analysis of microalgae production. *Biofuels from Algae (Second Edition)*: 551-566 (2019).

55. Oostlander PC, van Houcke J, Wijffels RH and Barbosa MJ, Microalgae production cost in aquaculture hatcheries. *Aquaculture* **525**: 735310 (2020).

56. El-Sheekh MM, El-Shouny WA, Osman MEH and El-Gammal EWE, Growth and heavy metals removal efficiency of *Nostoc muscorum* and *Anabaena subcylindrica* in sewage and industrial wastewater effluents. *Environmental Toxicology and Pharmacology* **19**: 357-365 (2005).

57. Kumar KS, Dahms H-U, Won E-J, Lee J-S, Shin K-H, Microalgae–A promising tool for heavy metal remediation. *Ecotoxicology and Environmental Safety* **113**: 329-352 (2015).

58. Lu Q, Yang L and Deng X, Critical thoughts on the application of microalgae in aquaculture industry. *Aquaculture* **528**: 735538 (2020).

59. Arunakumara K and Zhang X, Heavy metal bioaccumulation and toxicity with special reference to microalgae. *J. Ocean Univ. China* **7**: 60-64 (2008).

60. Kumar KS, Dahms H-U, Won E-J, Lee J-S and Shin K-H, Microalgae - A promising tool for heavy metal remediation. *Ecotoxicology and Environmental Safety* **113**: 329-352 (2015).

61. Meng W, Wang Z, Hu B, Wang Z, Li H and Goodman RC, Heavy metals in soil and plants after long-term sewage irrigation at Tianjin China: A case study assessment. *Agricultural Water Management* **171**: 153-161 (2016).

62. Lu Q, Zhou W, Min M, Ma X, Chandra C, Doan YTT, Ma Y, Zheng H, Cheng S, Griffith R, Chen P, Chen C, Urriola PE, Shurson GC, Gislerød HR and Ruan R, Growing Chlorella sp. on meat processing wastewater for nutrient removal and biomass production. *Bioresource Technology* **198**: 189-197 (2015).

63. Lenters JD, Kratz TK and Bowser CJ, Effects of climate variability on lake evaporation: Results from a long-term energy budget study of Sparkling Lake, northern Wisconsin (USA). *Journal of Hydrology* **308**: 168-195 (2005).

64. Vallet-Coulomb C, Legesse D, Gasse F, Travi Y and Chernet T, Lake evaporation estimates in tropical Africa (Lake Ziway, Ethiopia). *Journal of Hydrology* **245**: 1-18 (2001).

65. Chen W, Song L, Gan N and Li L, Sorption, degradation and mobility of microcystins in Chinese agriculture soils: risk assessment for groundwater protection. *Environmental Pollution* **144**: 752-758 (2006).

66. Schaefer AM, Yrastorza L, Stockley N, Harvey K, Harris N, Grady R, Sullivan J, McFarland M and Reif JS, Exposure to microcystin among coastal residents during a cyanobacteria bloom in Florida. *Harmful Algae* **92**: 101769 (2020).

67. Do Nascimento M, Battaglia ME, Sanchez Rizza L, Ambrosio R, Arruebarrena Di Palma A and Curatti L, Prospects of using biomass of N_2-fixing cyanobacteria as an organic fertilizer and soil conditioner. *Algal Research* **43**: 101652 (2019).

68. Van Apeldoorn ME, Van Egmond HP, Speijers GJ, Bakker GJ, Toxins of cyanobacteria. *Molecular Nutrition & Food Research* **51**: 7-60 (2007).

69. Crush J, Briggs L, Sprosen J and Nichols S, Effect of irrigation with lake water containing microcystins on microcystin content and growth of ryegrass, clover, rape, and lettuce. *Environmental toxicology* **23**: 246-252 (2008).

70. Codd GA, Metcalf JS and Beattie KA, Retention of *Microcystis aeruginosa* and microcystin by salad lettuce (*Lactuca sativa*) after spray irrigation with water containing cyanobacteria. *Toxicon* **37**: 1181-1185 (1999).

71. Lu Q, Zhou W, Min M, Ma X, Ma Y, Chen P, Zheng H, Doan YTT, Liu H, Chen C, Urriola PE, Shurson GC and Ruan R, Mitigating ammonia nitrogen deficiency in dairy wastewaters for algae cultivation. *Bioresource technology* **201**: 33-40 (2016).

72. Bustillo-Lecompte CF and Mehrvar M, Slaughterhouse wastewater characteristics, treatment, and management in the meat processing industry: A review on trends and advances. *Journal of Environmental Management* **161**: 287-302 (2015).

73. Cheng SY, Show P-L, Lau BF, Chang J-S and Ling TC, New Prospects for Modified Algae in Heavy Metal Adsorption. *Trends in Biotechnology* **37**: 1255-1268 (2019).

74. Lu Q, Yang L and Deng X, Critical thoughts on the application of microalgae in aquaculture industry. *Aquaculture* **528**: 735538 (2020).

75. Cibis KG, Gneipel A and Koenig H, Isolation of acetic, propionic and butyric acid-forming bacteria from biogas plants. *Journal of Biotechnology* **220**: 51-63

(2016).

76. Schnurr PJ and Allen DG, Factors affecting algae biofilm growth and lipid production: A review. *Renewable and Sustainable Energy Reviews* **52**: 418-429 (2015).

77. Khawam G, Waller P, Gao S, Edmundson S, Wigmosta MS and Ogden K, Model of temperature, evaporation, and productivity in elevated experimental algae raceways and comparison with commercial raceways. *Algal Research* **39**: 101448 (2019).

78. Masood S, Zhao XQ and Shen RF, Bacillus pumilus promotes the growth and nitrogen uptake of tomato plants under nitrogen fertilization. *Scientia Horticulturae* **272**: 109581 (2020).

79. Cadez P and Nordlund S, The requirement for Mn2+ and Ca2+ in nitrogen fixation by the photosynthetic bacterium *Rhodospirillum rubrum*. *FEMS Microbiology Letters* **81**: 279-282 (1991).

80. Sun B, Bai Z, Bao L, Xue L, Zhang S, Wei Y, Zhang Z, Zhuang G and Zhuang X, *Bacillus subtilis* biofertilizer mitigating agricultural ammonia emission and shifting soil nitrogen cycling microbiomes. *Environment International* **144**: 105989 (2020).

81. Sun B, Gu L, Bao L, Zhang S, Wei Y, Bai Z, Zhuang G and Zhuang X, Application of biofertilizer containing *Bacillus subtilis* reduced the nitrogen loss in agricultural soil. *Soil Biology and Biochemistry* **148**: 107911 (2020).

82. Iasimone F, Seira J, Desmond-Le Quemener E, Panico A, De Felice V, Pirozzi F and Steyer J-P, Bioflocculation and settling studies of native wastewater filamentous cyanobacteria using different cultivation systems for a low-cost and easy to control harvesting process. *Journal of Environmental Management* **256**: 109957 (2020).

83. Xu X, Gu X, Wang Z, Shatner W and Wang Z, Progress, challenges and solutions of research on photosynthetic carbon sequestration efficiency of microalgae. *Renewable and Sustainable Energy Reviews* **110**: 65-82 (2019).

84. Lu Q, Zhou W, Min M, Ma X, Chandra C, Doan YTT, Ma Y, Zheng H, Cheng S,

Griffith R, Chen P, Chen C, Urriola PE, Shurson GC, Gislerod HR and Ruan R, Growing *Chlorella* sp on meat processing wastewater for nutrient removal and biomass production. *Bioresource technology* **198**: 189-197 (2015).

85. Daneshvar E, Antikainen L, Koutra E, Kornaros M and Bhatnagar A, Investigation on the feasibility of *Chlorella vulgaris* cultivation in a mixture of pulp and aquaculture effluents: Treatment of wastewater and lipid extraction. *Bioresource technology* **255**: 104-110 (2018).

86. Rueda E, Jesus Garcia-Galan M, Ortiz A, Uggetti E, Carretero J, Garcia J and Diez-Montero R, Bioremediation of agricultural runoff and biopolymers production from cyanobacteria cultured in demonstrative full-scale photobioreactors. *Process Safety and Environmental Protection* **139**: 241-250 (2020).

87. Tejido-Nunez Y, Aymerich E, Sancho L and Refardt D, Treatment of aquaculture effluent with *Chlorella vulgaris* and *Tetradesmus obliquus*: The effect of pretreatment on microalgae growth and nutrient removal efficiency. *Ecological Engineering* **136**: 1-9 (2019).

88. Gorain PC, Paul I, Bhadoria PS and Pal R, An integrated approach towards agricultural wastewater remediation with fatty acid production by two cyanobacteria in bubble column photobioreactors. *Algal Research-Biomass Biofuels and Bioproducts* **42**: 101594 (2019).

89. Cheng P, Chen D, Liu W, Cobb K, Zhou N, Liu Y, Liu H, Wang Q, Chen P, Zhou C and Ruan R, Auto-flocculation microalgae species *Tribonema* sp. and *Synechocystis* sp. with T-IPL pretreatment to improve swine wastewater nutrient removal. *Science of the Total Environment* **725**: 138263 (2020).

90. He Y, Wang R, Liviu G and Lu Q, An integrated algal-bacterial system for the bio-conversion of wheat bran and treatment of rural domestic effluent. *Journal of Cleaner Production* **165**: 458-467 (2017).

91. Guidhe A, Ansari FA, Singh P and Bux F, Heterotrophic cultivation of microalgae using aquaculture wastewater: A biorefinery concept for biomass production and nutrient remediation. *Ecological Engineering* **99**: 47-53 (2017).

92. Wu X, Cen Q, Addy M, Zheng H, Luo S, Liu Y, Cheng Y, Zhou W, Chen P and Ruan R, A novel algal biofilm photobioreactor for efficient hog manure wastewater utilization and treatment. *Bioresource technology* **292**: 121925 (2019).

93. Wang L, Li Y, Chen P, Min M, Chen Y, Zhu J and Ruan RR, Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella* sp. *Bioresource technology* **101**: 2623-2628 (2010).

94. Ekinci K, Erdal I, Uysal O, Uysal FO, Tunce H and Dogan A, Anaerobic Digestion of Three Microalgae Biomasses and Assessment of Digestates as Biofertilizer for Plant Growth. *Environmental Progress & Sustainable Energy* **38**: e13024 (2019).

95. Garcia-Gonzalez J and Sommerfeld M, Biofertilizer and biostimulant properties of the microalga *Acutodesmus dimorphus*. *Journal of Applied Phycology* **28**: 1051-1061 (2016).

96. Grzesik M and Romanowska-Duda Z, Improvements in Germination, Growth, and Metabolic Activity of Corn Seedlings by Grain Conditioning and Root Application with Cyanobacteria and Microalgae. *Polish Journal of Environmental Studies* **23**: 1147-1153 (2014).

97. Schreiber C, Schiedung H, Harrison L, Briese C, Ackermann B, Kant J, Schrey SD, Hofmann D, Singh D, Ebenhoeh O, Amelung W, Schurr U, Mettler-Altmann T, Huber G, Jablonowski ND and Nedbal L, Evaluating potential of green alga *Chlorella vulgaris* to accumulate phosphorus and to fertilize nutrient-poor soil substrates for crop plants. *Journal of Applied Phycology* **30**: 2827-2836 (2018).

98. Siebers N, Hofmann D, Schiedung H, Landsrath A, Ackermann B, Gao L, Mojzes P, Jablonowski ND, Nedbal L and Amelung W, Towards phosphorus recycling for

agriculture by algae: Soil incubation and rhizotron studies using P-33-labeled microalgal biomass. *Algal Research-Biomass Biofuels and Bioproducts* **43**: 101634 (2019).

99. de Caire GZ, de Cano MS, Palma RM and de Mule CZ, Changes in soil enzyme activities following additions of cyanobacterial biomass and exopolysaccharide. *Soil Biology & Biochemistry* **32**: 1985-1987 (2000).

100. Horácio EH, Zucareli C, Gavilanes FZ, Yunes JS, dos Santos Sanzovo AW and Andrade DS, Co-inoculation of rhizobia, azospirilla and cyanobacteria for increasing common bean production. *Semina: Ciências Agrárias* **41**: 2015-2028 (2020).

101. Nisha R, Kiran B, Kaushik A and Kaushik CP, Bioremediation of salt affected soils using cyanobacteria in terms of physical structure, nutrient status and microbial activity. *International Journal of Environmental Science and Technology* **15**: 571-580 (2018).

102. Zarezadeh S, Riahi H, Shariatmadari Z and Sonboli A, Effects of cyanobacterial suspensions as bio-fertilizers on growth factors and the essential oil composition of chamomile, *Matricaria chamomilla* L. *Journal of Applied Phycology* **32**: 1231-1241 (2020).

103. Mutale-joan C, Redouane B, Najib E, Yassine K, Lyamlouli K, Laila S, Zeroual Y and Hicham EA, Screening of microalgae liquid extracts for their bio stimulant properties on plant growth, nutrient uptake and metabolite profile of *Solanum lycopersicum* L. *Scientific Reports* **10**: 2820 (2020).

104. Kholssi R, Marks EAN, Minon J, Montero O, Debdoubi A and Rad C, Biofertilizing Effect of *Chlorella sorokiniana* Suspensions on Wheat Growth. *Journal of Plant Growth Regulation* **38**: 644-649 (2019).

105. Wake H, Akasaka A, Umetsu H, Ozeki Y, Shimomura K and Matsunaga T, Enhanced germination of artificial seeds by marine cynobacterial extract. *Applied Microbiology*

& Biotechnology **36**: 684-688 (1992).

106. Grzesik M, Romanowska-Duda Z and Kalaji HM, Effectiveness of cyanobacteria and green algae in enhancing the photosynthetic performance and growth of willow (*Salix viminalis* L.) plants under limited synthetic fertilizers application. *Photosynthetica* **55**: 510-521 (2017).

Tables

Table 1. Algae-based nutrients recovery from agriculture-related wastewater

Wastewater	Algal strain	Nutrients removal efficiency (%)			Biomass yield (g/L)	Reference
		COD	TN	TP		
Meat processing wastewater	*Chlorella* sp.	29.53-43.91%	49.48-50.94%	44.95-54.45%	1.40-1.54	84
Brewery wastewater	*Leptolyngbya* sp.	91.6%	89.4%	98.5%	0.74	6
Meat processing wastewater	*Scendesmus* sp. and *Chlorella* sp.	72-82%	75-80%	86-92%	*NA* *	7
Fish rearing wastewater	*Chlorella vulgaris*	75.48%	76.56%	92.72%	1.31	85
Agricultural runoff	Cyanobacteria	*NA*	95%	99%	*NA*	86
Aquaculture effluent	*Chlorella vulgaris*	*NA*	78.7%	94%	1.39	87
Dairy processing wastewater	*Chlorella vulgaris*	16.44-46.51%	42.58-64.98%	21.79-64.15%	*NA*	71
Agricultural runoff	*Anabaena sphaerica* & *Anabaena variabilis*	77.0-84.2%	~90%	~50%	~1.1	88
Swine wastewater	*Tribonema* sp.	49.7-55.6%	80.2-9.9%	66.4-72.7%	~1.4	89
Swine wastewater	*Synechocystis* sp.	62.7-68.6%	73.7-75.8%	71.4-71.6%	~1.6	
Rural wastewater	*Chlorella vulgaris*	69.17-88.78%	62.04-82.49%	*NA*	1.34	90
Fish rearing wastewater	*Chlorella sorokiniana*	71.88%	75.56-84.51%	73.35%	1.15-4.02	91
Aquaculture effluent medium	*Monoraphidium* sp. & *Scenedesmus* sp.	*NA*	82-98%	66-90%	0.6-1.2	21

Hog manure	*Chlorella vulgaris*	95.67%	69.55%	64.40%	7.37 g/m^2	92
Digested dairy manure	*Chlorella* sp.	27.4–38.4%	75.7–82.5%	62.5–74.7%	1.47-1.71	93
Cattle manure	*Scenedesmus* sp.	over 90%	over 90%	79-88%	2-4	20

* "*NA*" refers to "not available"

Table 2. Effects of algal bio-fertilizer on plants growth and soil quality

Algal bio-fertilizer	Algal species	Pant or crop	Parameters	Main conclusion	Reference
Slow-release bio-fertilizer	*Chlorella* sp., *Neochloris conjuncta* & *Botryococcus braunii*	Corn plant	Plant: dry weight, metal (Fe, Zn, Mn & Cu) content, uptake of macro-elements (N, P, K, Ca, & Mg)	The use of digested *Chlorella* sp. at 5 t ha^{-1} promotes plant growth and increases the contents of metals in corn plant.	94
	Acutodesmus dimorphus	Roma tomato plant	Plant: numbers of lateral root, flower bud, and branch; total fresh plant weight	Algal fertilizer enhances plant growth and floral production.	95
	Nannochloropsis oculata	Tomato plant and fruit	Plant: leaf length and weight, metal (K, Ca, Mg, Zn, Mn, Fe, & Cu) content Fruit: fruit yield, contents of sugars and carotenoids	(1) With regard to plant growth, algal biomass can replace conventional fertilizer. (2) Algal fertilizer improves fruit quality by increasing the contents of sugars and carotenoids in tomato fruits.	33
	Chlorella sp.	Corn seed	Seed: germination rate and	(1) Algal bio-fertilizer increases the	96

	and plant	germination period Plant: length of roots and leaves, weight of roots and leaves, photosynthesis activity	germination rate of seeds and shortens germination period. (2) Algal fertilizer promotes the growth of roots and leaves and enhances photosynthesis.	
Chlorella vulgaris	Wheat Plant	Plant: weights of shoot and root, root density, length, and diameter;	Algal biomass is a viable option for delivering nutrients to support agriculture on marginal soils.	97
Chlorella vulgaris & *Spirulina platensis*	Rice plant	Plant: plant height, number of leaves per plant, plant weight, leaves area per plant, seed yield characters Soil: soil biological activity (CO_2 evolution, dehydrogenase activity, nitrogenase activity, etc.), soil chemical properties (pH, available-N, available-P, available-K)	(1) *Chlorella vulgaris* and *Spirulina platensis* can be used as bio-fertilizer to enhance rice yield. (2) Algal bio-fertilizer improves the biological and chemical properties of the soil.	30
Tetraselmis sp.	Date palm	Plant: numbers of roots and leaves, shoot length, stem thickness chlorophyll concentration Soil: total N, total P, total K	The addition of appropriate amount of algal biomass in soil promotes plant growth, improves the elemental composition of soil and maintains a safe low level of heavy metals in soil.	29
Nannochloropsi	*NA* *	Soil: content of organic carbon,	(1) Algal biomass after lipid extraction can	37

	s salina		microbial biomass carbon, total N, extractable inorganic N, etc.	be used as a soil amendment for agricultural production. (2) At high addition rates, problems with excess soil salinity and sodicity may occur.	
	Chlorella vulgaris	Wheat plant	Soil: P content in soil	(1) P release from algal biomass increases the concentrations of labile and moderately labile P fractions in soil. (2) Algal fertilizer releases P when incorporated into the soil to support or even sustain plant nutrition	98
Cyanobacteria	*Nostoc muscorum* & *Tolypothrix tenuis*	*NA*	Soil: activities of enzymes (β-glucosidase, urease, arylsulphatase, protease, etc.)	Cyanobacterial biomass and exopolysaccharide result in an increase of enzymatic activities	99
	Anabaena cylindrica	Common bean	Plant: plant height, number of nodules, nodule dry matter, shoot dry matter, accumulated shoot nitrogen, number of pods per plant, number of grains per pod, hundred grain weight, grain plant weight	Bio-fertilizer increases nodulation, plant growth, and production of the common bean.	100
	Consortia of *Nostoc ellipsosporum*	Pearl millet & wheat	Soil: nutrient dynamics in soil, microbial activities, physical characteristics (bulk density,	(1) Cyanobacteria perform well in bioameliorating salt-affected semi-arid soils. (2) Grain yield and leaf area are	101

and *Nostoc punctiforme*		water holding capacity, etc.) Plant: leaf area, spike length, grain yield, protein content	improved.	
Nostoc sp. and *Anabaena* sp.	*NA*	Soil: formation of soil surface consortia, biomass adherence to soil under water flush treatment	Cyanobacteria promote the formation of soil surface consortia and improve surface stabilization of agricultural soil	14
Oscillatoria sp., *Nostoc* sp. and *Scytonema* sp.	*NA*	Soil: soil microbiota, contents of available nutrients (P, K, Na, Ca & Mg)	Cyanobacteria improve crust formation, favor the proliferation of other microorganisms, and restore microbial populations in soil	15
Leptolyngbya sp., *Oscillatoria* sp., *Microcoleus vaginatus*, *Nostoc commune*, etc.	*NA*	Soil: soil physicochemical properties and soil stability parameters	(1) Cyanobacteria are able to colonize soils from arid and semi-arid areas. (2) Extracellular polymeric substances secreted by cyanobacteria blind soil particle together, increasing surface stability and reducing clay dispersion.	31
Nostoc carneum, *Wollea vaginicola*, & *Nostoc punctiforme*	Chamomile	Plant: weights of shoot and root, lengths of shoot and root, flower head diameter and weight, weight of essential oil Soil: contents of nutrients (Ca, P, & N)	(1) Cyanobacteria promote the plant growth and increase the weight of essential oil. (2) *Wollea vaginicola* dramatically increases the P content in soil	102

	Nostoc sp. & *Oscilatoria* sp.	*NA*	Soil: biological soil crust quality indicators, soil loss	Inoculation of cyanobacteria lead to the biological soil crust formation and prevent the soil loss	35
Liquid bio-fertilizer	Liquid extract of cyanobacteria, Chlorophyta, Rhodophyta, etc.	Tomato plant	Plant: root length, root weight, shoot length, shoot weight, root concentrations of N, P and K	Liquid extracts of algae stimulate plant growth, enhance nutrient uptake, and change metabolite profile of tomato	103
	Acutodesmus dimorphus extract	Tomato seed and plant	Seed: germination rate Plant: numbers of lateral root, flower bud, and branch; total fresh plant weight	Algal extract triggers faster seed germination and results in greater numbers of flowers and branches per plant	95
	Chlorella sorokiniana suspension	Wheat plant	Plant: lengths of roots and shoots, fresh weights, and dry weights	Algal extract increases lengths of roots and shoots, fresh weights, and dry weights of wheat plant	104
	Marine cyanobacterial extract (*Synechococcus* sp.)	Carrot cell (seed)	Seed: germination rate	Algal extract increases the germination rate of artificial seeds	105
	Microcystis aeruginosa,	Willow plant	Plant: height, fresh mass, number of shoots per plant,	Foliar application of algal fertilizer promotes plant growth and biomass yield	106

Anabaena sp., and *Chlorella* sp.		index of chlorophyll content in leaves, activity of RNase and total dehydrogenases	and increases chlorophyll content in leaves and photosynthetic rate	
Chorococcum sp. extract	*Vigna radiata*, *Solanum lycopersicum*, *Cucumis sativus* & *Capsicum annuum*	Plant: numbers of leaves and roots, lengths of root and shoot, biochemical parameters (total protein, lipid, carbohydrate, phenol), contents of pigments (total chlorophyll and carotenoids)	(1) A maximum growth rate is found in 20% algal liquid fertilizer. (2) Algal liquid fertilizer at higher concentration inhibits the growth and results in lower biochemical content.	32

* "*NA*" refers to "not available"

Table 3. Calculation parameters of algal biomass production in wastewater

	Nitrogen (N)		Phosphorus (P)	
Maximum percentage (%)	5.87		0.95	
Productivity ($g/m^3/day$)	Maximum	Minimum	Maximum	Minimum
	1.057	0.329	0.171	0.053
Volume of wastewater (m^3)	9.464×10^5	3.042×10^6	5.848×10^6	1.880×10^7

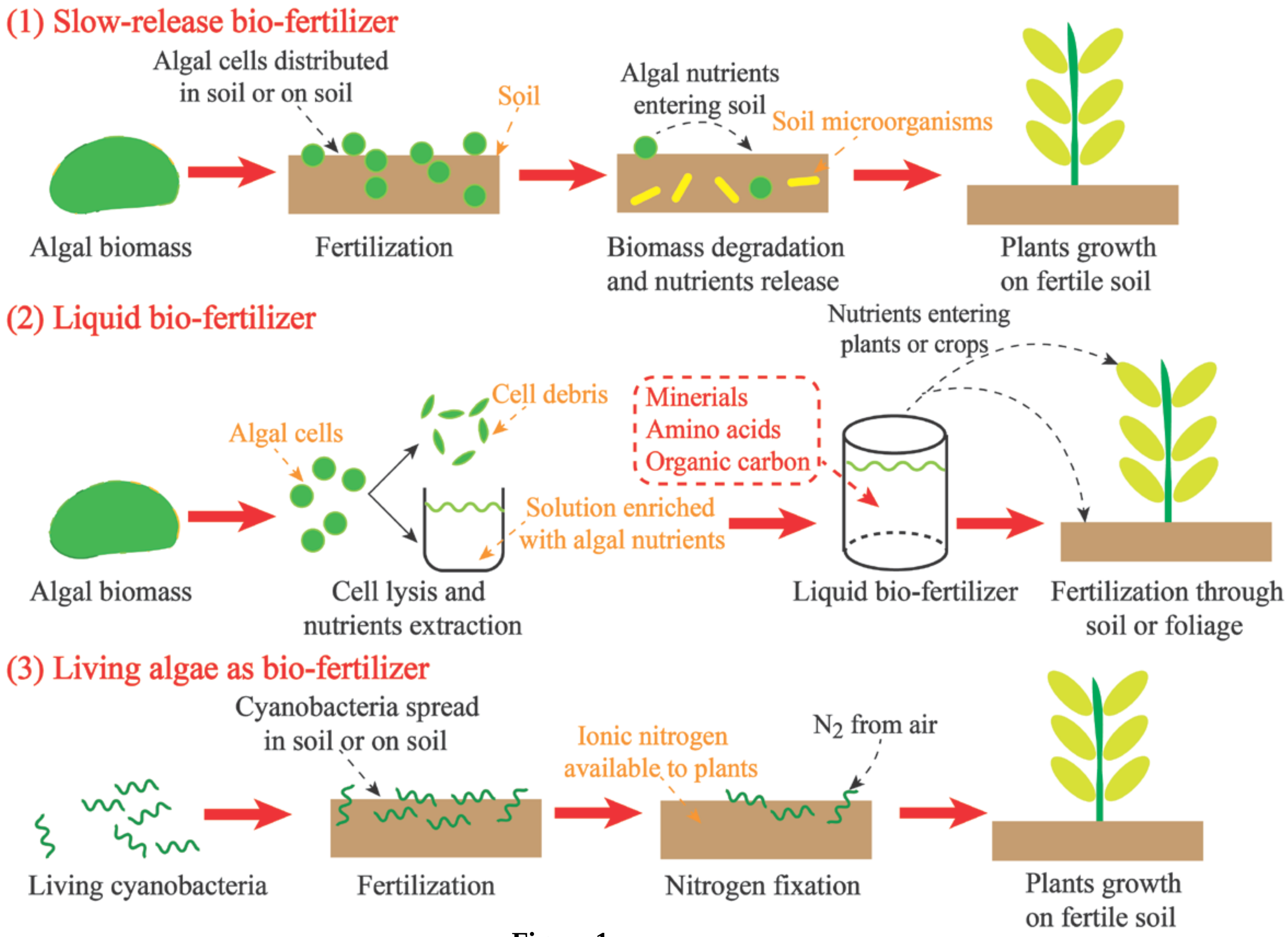
Area of algae culture system* (m^2)	1.893×10^6	6.084×10^6	11.696×10^6	37.594×10^6
Area of algae culture system* (km^2)	1.893	6.084	11.696	37.594

* Open raceway ponds with 0.5 m depth are applied for algae culture and wastewater remediation.

Figure captions

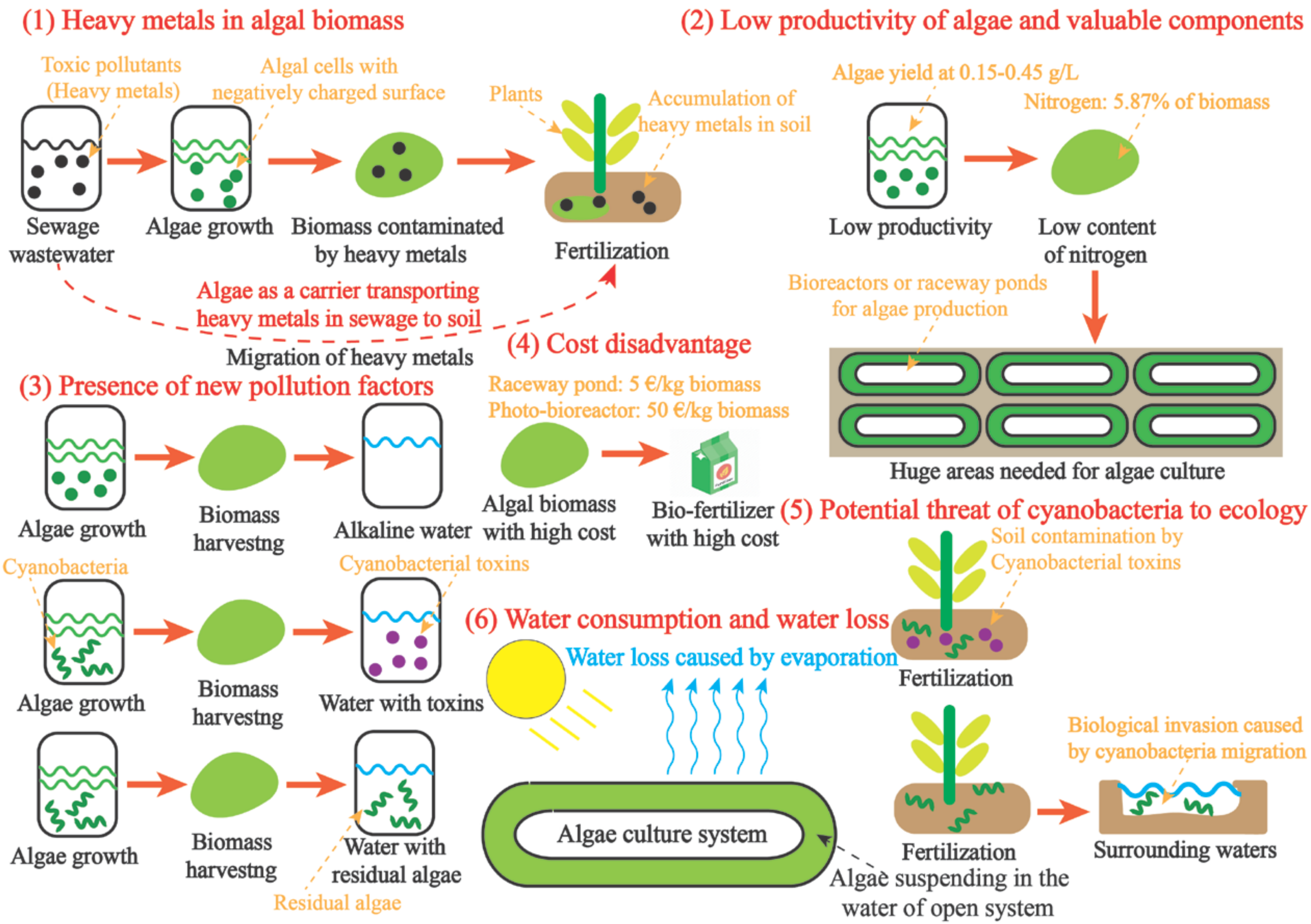
Figure 1. Three models of utilizing algal bio-fertilizers in agriculture

Figure 2. Technical defects of algae-based wastewater remediation for bio-fertilizer production



(1) Slow-release bio-fertilizer
Algal cells distributed in soil or on soil
Soil
Algal nutrients entering soil
Soil microorganisms
Algal biomass
Fertilization
Biomass degradation and nutrients release
Plants growth on fertile soil
(2) Liquid bio-fertilizer
Nutrients entering plants or crops
Cell debris
Minerials
Amino acids
Organic carbon
Algal cells
Solution enriched with algal nutrients
Algal biomass
Cell lysis and nutrients extraction
Liquid bio-fertilizer
Fertilization through soil or foliage
(3) Living algae as bio-fertilizer
Cyanobacteria spread in soil or on soil
Ionic nitrogen available to plants
N2 from air
Living cyanobacteria
Fertilization
Nitrogen fixation
Plants growth on fertile soil

Figure 1



(1) Heavy metals in algal biomass
Toxic pollutants (Heavy metals)
Algal cells with negatively charged surface
Plants
Accumulation of heavy metals in soil
Sewage wastewater
Algae growth
Biomass contaminated by heavy metals
Fertilization
Algae as a carrier transporting heavy metals in sewage to soil
Migration of heavy metals
(2) Low productivity of algae and valuable components
Algae yield at 0.15-0.45 g/L
Nitrogen: 5.87% of biomass
Low productivity
Low content of nitrogen
Bioreactors or raceway ponds for algae production
Huge areas needed for algae culture
(3) Presence of new pollution factors
Algae growth
Biomass harvestng
Alkaline water
Cyanobacteria
Cyanobacterial toxins
Algae growth
Biomass harvestng
Water with toxins
Algae growth
Biomass harvestng
Water with residual algae
Residual algae
(4) Cost disadvantage
Raceway pond: 5 €/kg biomass
Photo-bioreactor: 50 €/kg biomass
Algal biomass with high cost
Bio-fertilizer with high cost
(5) Potential threat of cyanobacteria to ecology
Soil contamination by Cyanobacterial toxins
Fertilization
Biological invasion caused by cyanobacteria migration
Fertilization
Surrounding waters
(6) Water consumption and water loss
Water loss caused by evaporation
Algae culture system
Algae suspending in the water of open system

Figure 2