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REVIEW

Biologia Futura: potential of different forms of microalgae for soil improvement

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Abstract

Products derived from microalgae have great potential in diverse field. As a part of the enhancing agriculture application, various forms of microalgae applications have been developed so far. They are known to influence soil properties. The various forms of application may enhance soil in more or less similar manner. They can help improve soil health, nitrogen, and phosphorus content, and even carbon sequestration. Thus, overall, it can enhance fertility of the soil.

Keywords Microalgae · Soil properties · Carbon sequestration

Introduction

Microalgae have captivated attention of many researchers in recent years due to their potential wide range of application (Spolaore et al. 2006). Some researcher mainly studies the production of biodiesel and other biofuels (bioethanol, biomethane, and biohydrogen) and generation of heat and electricity (Brennan and Owende 2010). While many studies the development of high value-added products from microalgae in areas such as nutrition and human health, aquaculture, cosmetics, and biofertilizers (Borowitzka. 2013). One of such implementations of Microalgae is in the field of agriculture considering the carbon and nitrogen fixing ability of some species.

Agricultural applications

Species like the Chlorophyte microalga has been proven to stabilize the soil and alters the hydrological properties (e.g., water retention) of crust covered soils in arid and semi-arid environments (Evans and Johansen 1999; Belnap and Lange 2003). Biological capture of carbon dioxide by using microalgae has shown promising, as microalgae fix CO_2 during their growth (Wang et al. 2008; Douskova et al. 2009). Therefore, activities of microalgae improving soil functions and properties might be enhanced and exploited through applications of algal biomass as biofertilizers.

Apart from functions improving soil health, soil applications of microalgae may also serve for mitigation or sequestration of atmospheric carbon dioxide. Though autotrophic microorganisms are not generally thought to have a keyhole in CO_2 fixation and sequestration in soils, global net carbon uptake of cryptogamic covers from the atmosphere amounts to ~3.9 Pg(Petagram) year^{-1}, which is on a scale similar to the global annual carbon release due to biomass burning and fossil-fuel combustion, respectively (Elbert et al. 2012; Yuan et al. 2012).

Furthermore, microalgae are nutrient-rich, can store the inorganic nitrogen (N), Phosphorus (P) in excess within the cells in the form of protein and polyphosphate (Solovchenko et al. 2016) and thus own potential to transformed from bio-waste to biofertilizer (Ray et al. 2013; Mukherjee et al. 2015; Santos and Pires, 2018.). *Chlorella*, a green microalga, contains significant quantities of N and P (up to 7–12% and 1–3% of their cell dry weight, respectively) (Powell et al. 2009; Cabanelas et al. 2013; Zhu et al. 2015). Hence, microalgae have the capacity to be used as cost effective, environmentally friendly, and sustainable alternative biofertilizer to traditional chemical fertilizer as they not only increase agricultural production but also minimizes the negative environmental impact on land use (Kawalekar 2013; Sigurnjak et al. 2017). Biofertilizer prevents loss of nutrients and can supply almost all the nutrients required for plant growth (Kokare

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et al. 2015) in the absences of chemical fertilizers, the crop also depends entirely on the mineralization of organically bound nutrients within the biofertilizers.

However, direct application of microalgae did not deliver significant difference on the growth of wheat (Schreiber et al. 2018) or rice (Ray et al. 2013; Mukherjee et al. 2015) because the dominant forms of the stored N and P in microalgae are proteins and polyphosphates that are difficult to decompose in soil and unable to be directly utilized by plants. So, several utility forms have been employed.

Microalgae forms for soil application

One such form is the hydrothermal carbonization (HTC), which can transform the microalgae biomass into hydrochars. This is recommended as it has been demonstrated to transform most polyphosphates and proteins from ligno-cellulosic feedstock (Funke et al. 2013; Kruse et al. 2016) and biosolids (Huang and Tang 2015; Huang et al. 2017;** Yu et al. 2019) into orthophosphate and ammonium or nitrate. HTC is cost-effective to avoid dehydrating the microalgae collected from the wastewater. Generally, biochar application can reduce soil pH and increase porosity, aeration, and redox potential, thus reducing NH_3. Citrate acid was added to increase the hydrochar yield (Heilmann et al. 2010) reduce hydrochar pH and promote the degradation of proteins and polyphosphates by acidic hydrolysis (Huang et al. 2017).

Another form of application is algal biofertilizer. Biofertilizers have emerged as best alternative to synthetic fertilizers. A biofertilizer comprises living microorganisms, which on application colonizes the rhizosphere or the interior of the plant, plant let or seed surfaces or soil, thus promoting growth by accelerating the availability of primary nutrients to the host plant. Biofertilizers comprise of microorganisms, including bacteria, fungi, cyanobacteria, and algae as well as their metabolites that can enhance soil, crop growth, and yield. Biofertilizers include symbiotic nitrogen fixers like *Rhizobium* spp. associated with leguminous crops, non-symbiotic free-living nitrogen fixers like Azotobacter, which can be used for crops like maize, wheat, cotton, mustard, potato, and other vegetable crops, while *Azospirillium* is mainly used for sorghum, millets, maize, sugarcane, and wheat. Algal biofertilizers like the cyanobacteria such as *Nostoc* sp., *Anabaena* sp., *Tolypothrix* sp., *Aulosira* sp. etc., have the potential to fix atmospheric nitrogen and are used in paddy fields. Some other types include mycorrhizae, organic fertilizers, and phosphate-solubilizing bacteria. *Pantoea agglomerans,* one of the phosphate-solubilizing bacteria such as strain P5, and *Pseudomonas putida* strain P13 can solubilize the insoluble phosphate from organic and inorganic sources. The *Azolla-Anabaena* and *Rhizobium* form the most important group of biofertilizers. Biofertilizers have numerous benefits to soil quality and crop yield as they enhance nutrient transfer, increase population of beneficial microorganisms, stabilize soil aggregates, and decrease reliance on fossil fuels. Benefits of different microalgal-biofertilizers are listed in Table 1.

A new emerging application is the harvest and utilization of algal biomass produced in waste treatment since microalgae may produce bioactive substances such as phytohormones or accumulate elements of interest (Mallick 2002; Stirk and Van Staden 2010). Aerobic soil applications with living algae present an entirely different scenario: microalgae are specifically adapted to aquatic environments, requiring continuous hydration for maintenance of cellular structure, nutrient acquisition, and gas diffusion. As such, in soil environments, the proliferation of microalgae is strongly conditional by humidity, and the temporal desiccation will occur in arid and semi-arid environments where the benefits of soil organic matter may be greatest. Despite these obvious

Table 1 Biofertilizer and their contributions

Major Class of microalgae Biofertilizer	Species name	Contribution	References
Blue-green algae	*Nostoc*, *Anabaena*, *Aulosira*, *Tolypothrix*, *Nodularia*, *Cylindrospermum*, *Scytonema*, *Aphanothece*, *Calothrix*, *Anabaenopsis*, *Mastigocladus*, *Fischerella*, *Stigonema*, *Haplosiphon*, *Chlorogloeopsis*, *Camptylonema*, *Gloeotrichia*, *Nostochopsis*, *Rivularia*, *Schytonematopsis*, *Westiella*, *Westiellopsis*, *Wollea*, *Plectonema* and *Chlorogloea*	(1) Fix 18–45 kg N/ha in submerged rice field. (2) Produce growth-promoting substances	Singh (2008) Watanabe and Cholikul (1979) Vaishampayan (1998) De (1939)
Anabaena Azolla association	*Anabaena azollae*	(1) Fixes 40–80 kg N/ha (2) Used as green manure because of large biomass	Vaishampayan et al. (2001) Moore (1969)

Source: Chatterjee et al. (2017)

constraints, microalgae, alone or as a component of biological soil crusts (BSC) play roles in soil nutrient cycling and water fluxes (Maestre et al. 2011).

The de-oiled microalgal biomass waste (DOMBW), which contains a high amount of nitrogen, phosphorus, potassium, and other nutrients, can serve as a biofertilizer (Dineshkumar et al. 2018). Microalgae-based biofertilizer has the capability to decrease nutrient losses through a consistent release of nutrient, which can suitably fulfil the nutrient requirements of the crops (Renuka et al. 2018). Besides macronutrients, microalgae also contain trace elements and natural phytohormones, which are essential for proper growth and development of plants (Dineshkumar et al. 2018). Using N-rich biomass of microalgae as biofertilizer also have additional benefits such as carbon sequestration, improved soil health, soil water retention, stability of soil aggregates, and prevention of nutrient losses (Sole-Bundo et al. 2017) therefore, DOMBW is also a promising source of biofertilizer.

Implemented in photobioreactors, microalgae can metabolize and accumulate residual nutrients from agro-industrial waste (Morales-Amaral et al. 2015). They have potential to increase soil organic matter content and fix additional atmospheric C into Croplands need to be tested. Soil organic matter can correlate with P adsorption both positively mainly due to the anionic character of organic matter, and negatively, blocking P adsorption sites in soil (Novais et al. 2007). Therefore, it should be stated that each material, whether animal waste, biochar, or any biomass rich in organic matter may, by virtue of its constitution behave differently when added to the fertilizer mass. In this context, the possibility of microbial biomass grown in wastewaters may also be added to phosphate fertilizers to increase the adsorption efficiency of P by plants. There are few published works referring to these theme (Castro et al. 2017; Marks et al. 2017) and survey that evaluate the environmental impact of the use of this material as a source of nutrient are still incipient.

Different processes of preparation

1. Microalgae Biofertilizer (Castro et al. 2020):

   To be used as biofertilizer, they need to undergo two production steps, viz. cultivation, and processing. They are explained as below:

   Algae Biomass cultivation:

   (a) Multiplication

   A High-Rate Algal Pond (HRAP) (area = 3.30 m^2 & volume=1 m^3) was operated in batch mode (14 days of operation) to produce MB. The HRAP had a six-blade stainless steel paddlewheel, powered by a 1 HP electric motor responsible for operating 12 ponds. During the operation, the CO_2 supplementation was controlled from the pH variation in the units. In addition, it had a CO_2 injection system, in which a gas cylinder injection system, in which a gas cylinder containing 99% CO_2 and a pump were used to recirculate the effluent in the carbonation column.

   (b) Coagulation

   After the production phase, the biomass was treated with 505 mV s^{-1}(millivolt per second) sodium hydroxide (NaOH), promoting a pH increase up to 12. And then for coagulation, a suitable hydraulic gradient was generating by moving a paddlewheel for nearly 2 h.

   (c) Collection

   Biomass was collected after resting the effluent inside the HRAP for 24h. The main characteristics of the biomass at the end of the batch were as follows: volatile suspended soils = 571.81 mg L^{-1}(milligram per litre); total phosphorus = 7.40 mg L^{-1} and total nitrogen = 68.40 mg L^{-1}.

   (d) Drying

   The biomass was dried in a forced circulation greenhouse (3 kwh) for 2 days. The greenhouse has drying capacity up to 400 kg of biomass at a time.

Biofertilizer processing

The biofertilizer was produced by granulation process, with the addition of 12% dry MB (Microalgae Biomass) into triple superphosphate (TSP), $Ca(H_2PO_4)_2.H_2O$. This proportion was chosen, after previous experiment conducted by Castro et al. 2020. Authors tested several other addition proportions and 12% of MB corresponds to the value that presented higher P content in the millet plant shoot (*Pennisetum glaucum* L.) TSP + 12% MB showed no difference in P diffusion in the soil, while increase in proportion above 30% MB clearly impaired P diffusion.

(2) Hydrochar production from microalgae (Chu et al. 2020a, b):

In the processing, first the inoculum was maintained in 2L borosilicate bioreactors using sterilized medium, 3N-BBMV (Bold's Basal medium with vitamins and triple nitrate).

Operational conditions were as follows: constant aeration using 2.5% CO_2 at 0.2 Vvm (Volume of gas per volume of culture per minute), a photoperiod of 14:10 light: dark cycle, 150 µmol m^{-2} s^{-1} of luminance and temperature of 25 ± 1 °C.

The microalgal cells were then collected from a cultivation broth using centrifugation of 8000*g* for 5 min at 4 °C, followed by washing using distilled water and finally freeze-dried.

Hydrothermal carbonization (HTC) was conducted in a high-pressure (approximate 8 MPa, autogenerated during HTC) hydrothermal reactor. The process begins by loading the microalgae into the reactor. The reactor was sealed and heated at 260 °C for 1 h and then allowed to naturally cool down to room temperature overnight. The solid hydrochars produced by HTC were collected by centrifugation and dried at 70 °C until no further weight loss. Two hydrochars that had been produced by using different reaction media viz. CVHW, *Chlorella vulgaris*-derived hydrochars with water (employing deionized water) and CVHCA *Chlorella vulgaris*-derived hydrochars with citric acid (employing 1 wt% citric acid). Citric acid was added to increase the hydrochar yield (Heilmann et al. 2010), reduce hydrochar pH and promote the degradation of proteins and polyphosphates by acidic hydrolysis (Huang et al. 2017).

(3) De-oiled microalgal biomass waste (DOMBW) (Nayak et al. 2019a, b):

The microalga was cultivated in an open raceway pond with a working volume of 60 L for 7 days in batch mode, where the solar intensities varied between 300 lx to 48000 lx. The temperature ranged from 27 °C to 33 °C and the average relative humidity varied from 55% to 92%. The culture was grown using domestic wastewater as the growth medium and supplemented with coal-fired flue gas (2.5% CO_2) as the carbon source. The different nutrient components present in the wastewater were as follows: ammonium (NH_4^+ –N) 38.6 mg L^{-1} Nitrate (NO_3^-–N) 17.1 mg L^{-1}, Phosphate (PO_4^{-3}–P) 9.24 mg L^{-1} and chemical oxygen demand (COD) 142.2 mg L^{-1}The flue gas comprised of CO_2 12% (v/v), carbon monoxides (CO) 0.55% (v/v) sulfur dioxides (SO_2) 0.3% (v/v) and nitrogen oxides (NOx) 61 ppm.

After cultivation, the culture was concentrated via flocculation using chitosan as flocculant. The upper medium layer was decanted, and the lower concentrated biomass phase was dried under direct sunlight. The organic cationic flocculant, chitosan was used to coagulate negatively charged microalgae cell to avoid high energy centrifugation method. The dried algal biomass is then used for the extraction of oil. the residual de-oiled biomass was dried at 70 °C for 48 h to remove the solvent, and it was stored at −80 °C until later use. For use as a fertilizer, the DOMBW was mixed into soil and used for the cultivation.

(4) Microalgae slurry (Marks et al. 2017):

Live algae collected or produced by earlier mentioned biomass production technique are used as a liquid slurry. No further processing is to be done. Those liquid slurry are used directly in field.

Influences of microalgae on soil properties

Even though the form of microalgae applied differs, their fate in soil is identical, i.e., their effect on the soil may be almost similar with negligible differences. So, their effect on soil has been clubbed together and discussed under the following subheadings.

Soil chemical properties

Soil pH is also known to be affected by algal application. Saha and Mandal (1979) reported an initial increase in soil pH, whereas contradictory to it Subhashini and Kaushik (1981) reported a significant reduction not only in pH but also in hydraulic conductivity, electrical conductivity (EC), and soil aggregation. cyanobacteria are also known for their ability to release trace elements from insoluble materials. Fe, Mn, and Zn are known to be influenced in rice fields by cyanobacterial growth (Das et al. 1991). Lange (1976) reported chelation of Fe, Cu, Mo, Zn, Co, and Mn through gelatinous sheath of many cyanobacterial species. This sheath is also known to reduce particle erosion and may adsorb charged nutrient cations (Whitton 2000). In summary, algal application influence soil properties through soil particle aggregation, phosphate and trace element release from insoluble minerals, and N storage and its slow release. The chlorella algae grown in an inert substrate can fix 0.5 mg of CO_2–C over the test period. *Chlorella* has greater effect of C fixation than the native algae.

Chlorella microalgae applied as powder or as hydrochar employing water or citrate solution, significantly improved the soil NH_4^+ concentration also improving NO_3–N concentration by 46.5%. Application of *Chlorella* in different forms affect the pH of soil, being consistent with pH of the material.

Nayak et al. (2016) reported a significantly lower pH value of the soil with supplementation of chemical fertilizer in comparison with those treated with de-oiled microalgae biomass waste (DOMBW). The increased value can be due to the release of NH_4^+–N form protein degradation of biomass. It was also found that supplementation of DOMBW results in significantly higher EC value of the soil in comparison to the application of other organic fertilizer at both tillering and harvesting stage of growth. High EC values shows continues availability of soluble nutrients in the form of both cations and anions to support healthy growth and development of plants especially rice (Eigenberg et al. 2002; Meng et al. 2018).

The nutrient availability was found to be optimum for proper growth of rice when soil was supplemented with algal-based fertilizer in comparison to chemical fertilizer or vermicompost supply. The likely explanation is that the DOMBW requires time to be decomposed into usable nutrients, which means that the nutrients are released steadily throughout the crop cultivation (Castro et al. 2017). A similar observation was also observed by Renuka et al. (2016), where available N, P, and K was found to be increased when microalgae was supplied.

Effect on soil physical properties

Effect of surface growth of inoculated cyanobacteria on subsurface properties of a brown earth, silt loam soil was studied by Rao and Burns (1991). Significant increase in soil polysaccharides, dehydrogenase, urease, and phosphatase activities was recorded. Improvement in soil aggregation was also seen; stable soil aggregates are essential to soil fertility. Studies of Burns and Davics (1986) suggested soil polysaccharides as major component responsible for soil stabilization. However, these effects were confined to surface layer of 0–0.7 cm depth. The results of Roychoudhury et al. (1979) also demonstrated improvement in soil aggregation.

Inoculations with cyanobacteria provide a better water holding capacity in the soil. These were supported by several other studies (Singh 2008; Bailey et al. 1973; de Winder et al. 1989). The improvement in soil aggregation was due to Algal proteoglycans which possess adhesive properties, and easily fasten cells to solid surfaces (Flaibani et al. 1989). Soil aggregation and arrangement of the soil aggregates are important as it directly affects temperature, aeration, and infiltration rates of the soil, which ultimately improves the physical environment of the crop (Falchini et al. 1996). Furthermore, besides soil aggregation, soil porosity is also enhanced by inoculating *Nostoc* strains on clay soils (Falchini et al. 1996). There are reports suggesting solubilization of insoluble forms of inorganic phosphate by cyanobacterial inoculation (Singh 2008; Kleiner and Harper 1977). It was further evidenced by studies of Bose et al. (1971), Cameron and Julian (1988), and Roychoudhury and Kaushik (1989), which advocated cyanobacterial phosphorous solubilizing activity on hydroxyapatite, tricalcium phosphate, and Mussorie rock phosphate. Apart from phosphorous, there are several evidences that witnessed an increase in N content and organic matter of soils inoculated with cyanobacteria (Singh and Singh 1989; Vaishampayan et al. 2001; Venkataraman 1993). Castro et al. (2020) studied that use of microalgae as biofertilizer along with chemical fertilizer is an efficient and viable option for improving and restoring soil fertility along with superior crop productivity.

Effect on soil biological properties

Although studies have been undergoing on the effect of algal biofertilizer on soil microflora, limited details are known about the associative changes in soil microbial community following inoculation with cyanobacteria or other algae. The application of a photosynthetic algal suspension increased eukaryotic and prokaryotic biomass and the activities of heterotrophic microorganisms in the soil. Rao and Burns (1991) reported an eightfold increase in bacterial members in the cyanobacteria inoculated columns, whereas increase in fungal population was not significant. Ibrahim et al. (1971) reported an increase in total microbial community in a pot experiment specifically nitrifiers (genera of *Azotobacter* and *Clostridium*) after inoculation of *Tolypothrix tenuis*. Acea et al. (2001) reported greater than four logarithmic unit increases in heterotrophic bacteria, actinomycetes, algal, and fungal propagules and three logarithmic unit increases in fungal mycelia after inoculating burnt soils with cyanobacteria. Similarly, Rogers and Burns (1994) reported a significant difference in the heterotrophic microbial population after inoculation of soil with *Nostoc muscorum*. These results suggest additional carbon and energy source due to cyanobacterial polysaccharides as one of the reasons behind increase in heterotrophic microbial populations. Increment in total nitrogen content of inoculated soil also stimulates indigenous soil microorganisms. Reports of Chu et al. (2020a, b) shows the application of *Chlorella vulgaris* powder and hydrochars had marked impacts on the activities of soil microorganisms that are responsible for nitrification and denitrification. Nutrient status of soil specifically nitrogen and phosphorous determines the mineralization of available carbon and thus affects the microbial community (Anderson and Gray, 1991).

Conclusions

Thus, from the above acknowledge information, we know that microalgae have high capability of improving the soil properties. Even though some findings are still uncertain like how much impact it can have on other microbial population under long-term application or how efficient it can be as compared with the inorganic sources. It is evident that the effect on soil properties has been successful so far though the results are based on short-term research. Since major farming population has already witness the immense impact of excessive use of chemicals on soil health leading to soil degradation, an organic supplement is needed to reduce its use. Addition of microalgae can be one such useful method to minimize the negative effects of excessive chemical use. Research on the application of microalgae in agriculture is still in its early stages and has yet to be tested on a large scale.

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Declarations

Conflict of interest The authors declare there is no conflict of interest.

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