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Original Article

Biochemical Characterization of Microalgal Biofertilizers for Nutrient Dynamics and Soil Fertility Enhancement

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Abstract
Microalgae are sustainable resources, with multiple applications in agriculture and offer a range of features that are specifically useful for overcoming the multifaceted agricultural scenario. This study aims to illustrate the recent developments in the field of microalgae biotechnology, specifically in relation to biofertilizers and to highlight ongoing research in this area and future research directions. The beneficial impact of microalgae on soil fertility, plant development and protection and even as an alternative to reduce the reliance on chemical fertilizers and pesticides can be attributed to the wide range of biomolecules produced in microalgae biomass, which include soluble amino acids, micronutrients, polysaccharides, phytohormones and other signaling molecules. In addition, the incorporation of microorganism biomass obtained from urban wastewater as biofertilizer promotes plant growth similar to other organic fertilizers or traditional manure; at the same time, it decreases the plant depletion of these nutrients in the soil. Furthermore, the role of microalgae obtained from wastewater treatment and utilization of biofertilizers, biofortifiers and biopesticides is considered a emerging topic / trending topic for the utilization of wastewater in agriculture. This study aims to identify different metabolites produced by microalgae that have a positive effect on plant growth and to understand their role in plant development and yield. Finally, the new challenges of promoting the sustainability of microalgae biomass through co-culture of algae and hydroponics and utilization of farm effluents are discussed and explored.

Keywords: *Microalgae Biotechnology, Biofertilizers, Soil Fertility, Plant Growth Promotion, Wastewater Utilization, Sustainability*

1. Introduction

By 2050 the global population is expected to reach 9.6 billion and their demand for food will continue to grow, resulting in the general application of intensive farming techniques, such as chemical fertilizers, pesticides and growth stimulants, to maximize crop yields; however, the ground will remain more or less disturbed by humans, with human-induced and influenced global change identified as a major driver of soil disturbance, with land use and implementation of farming practices, degradation, deposition of sulfur and nitrogen and contamination with heavy metals being the predominant drivers. In fact, when viewed in the context of the rising demand for food in the world, the growing environmental burden of increased agricultural intensification and the uncertain nature of change in our global climate, a need for sustainable alternatives to sustain and further increase agricultural output becomes apparent [1]. In this regard, microalgae are renewable resources that have diverse alternatives for agricultural applications [3] and they have been shown to have a set of capabilities of special relevance for the present complex agricultural application [3]. Additionally, microalgae biomass acts as a potential source of biomolecules such as soluble amino acids (AA), micronutrients, polysaccharides, phytohormones and other signaling molecules that boost soil fertility and plant growth and protection and can be used as alternatives to decrease the usage of chemical fertilizers and pesticides [6]. Furthermore, using the microalgae biomass as a biofertilizer significantly promoted plant growth similarly to that of other organic manures and inorganic fertilizers and reduced nutrient losses from the soil [4]. Biofertilizers are materials containing living microorganisms that colonize the rhizosphere or the inside of the plant and stimulate the growth of the plants by enhancing the availability of the primary nutrients to the plant when applied to seed, plant surfaces, or soil as per the FAO (2022) [5]. The use of microalgae for the treatment of wastewaters and the harvest of the valuable biomass as biofertilizer is an attractive idea [2].

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2. Literature Study

The biomass of microalgae contains many biomolecules such as soluble amino acids (AA), micronutrients, polysaccharides, phytohormones and other signaling molecules, which provide novel sources of plant growth promoters in turn to boost crop productivity and disease resistance [10]. Moreover, microalgae also improve the fertility of soil and help in the growth and protection of the plants and also serve as an alternative, reducing the dependency on chemical pesticides and fertilizers [8]. Microalgae are beneficial in soil nutrient cycling and stimulate plant growth by enhancing nutrient availability and biosynthesis of plant growth stimulators and bioactive compounds such as phytohormones [8]. Furthermore, the use of wastewater treatment-based microalgae and the application of generated biomass as biofertilizers, stimulants and pesticides have been greatly highlighted for implementation in the agricultural sector [7]. With the increasing need for food to ensure the food security of the hungry planet and to avoid the pollution of chemical fertilizers due to their excessive use in agriculture, biofertilizers became an essential part of the current agriculture [7]. According to this view, the aim of this study is to summarize the recent achievements in microalgae biotechnology related to biofertilizers, thus highlighting some of the current research in this area and future directions in development [7]. This study encompasses the elementary, basic and practical/sociological aspects of microalgae related to significant agricultural steps such as BNF, P cycling, impact on soil microorganisms, long-term effect on plants by phytohormones, direct association with plants, ong-term effect of soil nutrient cycling, biocontrol and soil stabilization [8]. In particular, use of the treated municipal wastewater as biofertilizer enhanced plant growth in a manner similar to that obtained by the different organic manures and inorganic fertilizers and reduced soil nutrient losses [9]. Moreover, microalgae biomass has the potential to be a sustainable alternative resource for reducing the current inorganic fertilizer use [9]. The current work seeks to explain the many plant growth-boosting metabolites generated by microalgae and their impact on plant growth and productivity [10]. Furthermore, introducing biofertilizers and biostimulants derived from microalgae (MBMAs) is increasingly becoming more popular, but such technologies are still not economically sustainable due to the nutrients and water requirements and energy-intensive downstream processes [10]. In this regard, we propose a circular economy approach towards bioremediation by using microalgae with biorefinery methods of producing high-value metabolites in addition to biofertilizer application [10]. Agricultural effluents and the co-culture of algae and hydroponics are investigated as innovative means aiming at a more sustainable biomass production of microalgae [10].

3. Materials and Methods

Axenic cultures of C. vulgaris (UTEX-26), S. platensis (UTEX-LB-2340) and Scenedesmus sp. The biomass was grown in Zarrouk or BG-11 medium under a 16:8 h light-dark cycle at 150 µmol $m^{-2}s^{-1}$ light intensity and 25 ± 2 °C and harvested during the stationary phase, lyophilized and ground to a fine powder. Extraction and quantification of proteins using the Lowry method, carbohydrates by phenol-sulfuric, lipids by using the Bligh-Dyer method, ash by heating at 550 °C, macronutrients such as TN (Kjeldahl method), TP (vanadomolybdate method) and TK (flame photometry) and EPS were extracted and quantified as glucose equivalents by HPLC-MS/MS for the quantification of phytohormones such as gibberellins, cytokinins and auxins. Soils were collected, air-dried, sieved (2 mm) and identified as sandy loam with initial pH 6.8 and organic C 1.2%. The treatments used are (i) (i) control (no amendment), (ii) chemical fertilizer [NPK 20:20:20 at 120 kg N ha^{-1}] and (iii–v) 1%, 2% and 5% (w/w) microalgal biofertilizer based on a 1:1:1 strain combination. Soils were kept in incubation at 25°C and 60% WHC and destructive sampling was conducted at 0, 15, 30, 60 and 90 days. Exchangeable K (flame photometry), accessible P (Olsen) and available N (Kjeldahl distillation) were determined. The enzyme activities measured were: catalase (permanganate titration), acid phosphatase (p-nitrophenyl phosphate), urease (indophenol) and dehydrogenase (TTC reduction). Microbial biomass carbon (MBC) by chloroform fumigation-extraction. Triplicate treatments were used and one-way ANOVA with Tukey's HSD ($p < 0.05$) was used to analyze the results.

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4. Results

1 Biochemical composition of the microalgal mixture

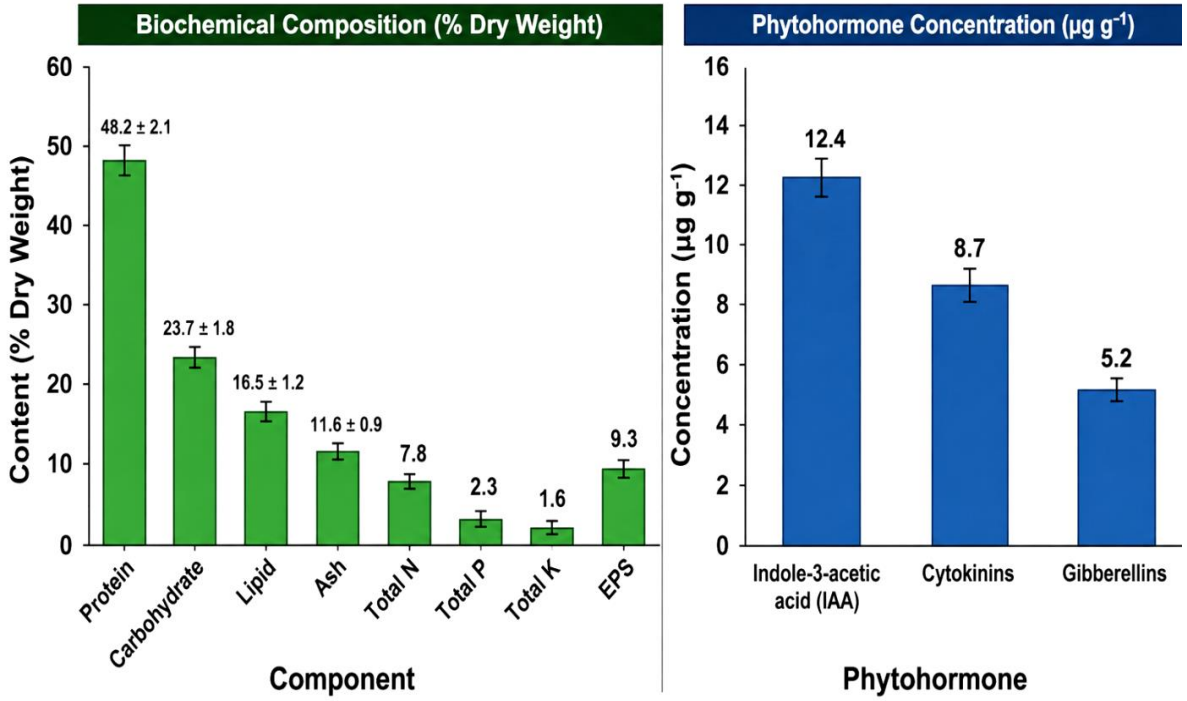

Figure 1: Growth Characteristics of Microalgal Strains

The high protein and N content in itself proves the biofertilizer for a delayed release N source. The EPS with the presence of phytohormones provides double biostimulant and soil conditioning forces.

2 Nutrient release dynamics

Table 1: Biochemical Composition and Phytohormone Content of Microalgal Strains

Treatment	Day 0	Day 30	Day 60	Day 90
Available N				
Control	28.4 ± 2.1	29.0 ± 1.9	28.2 ± 2.0	27.5 ± 1.8
Chemical fertiliser	28.6 ± 2.0	72.1 ± 3.8	58.4 ± 3.1	42.6 ± 2.9
BF 1%	28.5 ± 1.9	55.3 ± 3.0	62.8 ± 2.8	58.9 ± 2.6
BF 2%	28.3 ± 2.2	68.4 ± 3.5	79.2 ± 3.4	72.5 ± 3.0
BF 5%	28.7 ± 2.0	74.6 ± 3.9	88.5 ± 3.7	81.3 ± 3.2
Available P				
Control	12.1 ± 1.3	12.3 ± 1.1	11.9 ± 1.2	11.6 ± 1.0
Chemical fertiliser	12.0 ± 1.2	38.7 ± 2.5	29.4 ± 2.1	18.3 ± 1.7
BF 1%	12.2 ± 1.1	22.8 ± 1.9	28.5 ± 2.0	26.1 ± 1.8
BF 2%	12.1 ± 1.3	30.2 ± 2.2	36.7 ± 2.4	33.5 ± 2.2
BF 5%	12.3 ± 1.2	34.5 ± 2.7	42.1 ± 2.9	38.9 ± 2.5

In the case of chemical fertilizer, the data increase at day 30 and then fall sharply, whereas all BF treatments undergo a steady increase up to and at days 60–90 for N and 60 for P. However, the regulated mineralization of N and P is indicated by the 2% and 5% BF treatments being higher than the chemical treatment at day 60.

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3 Soil enzyme activities

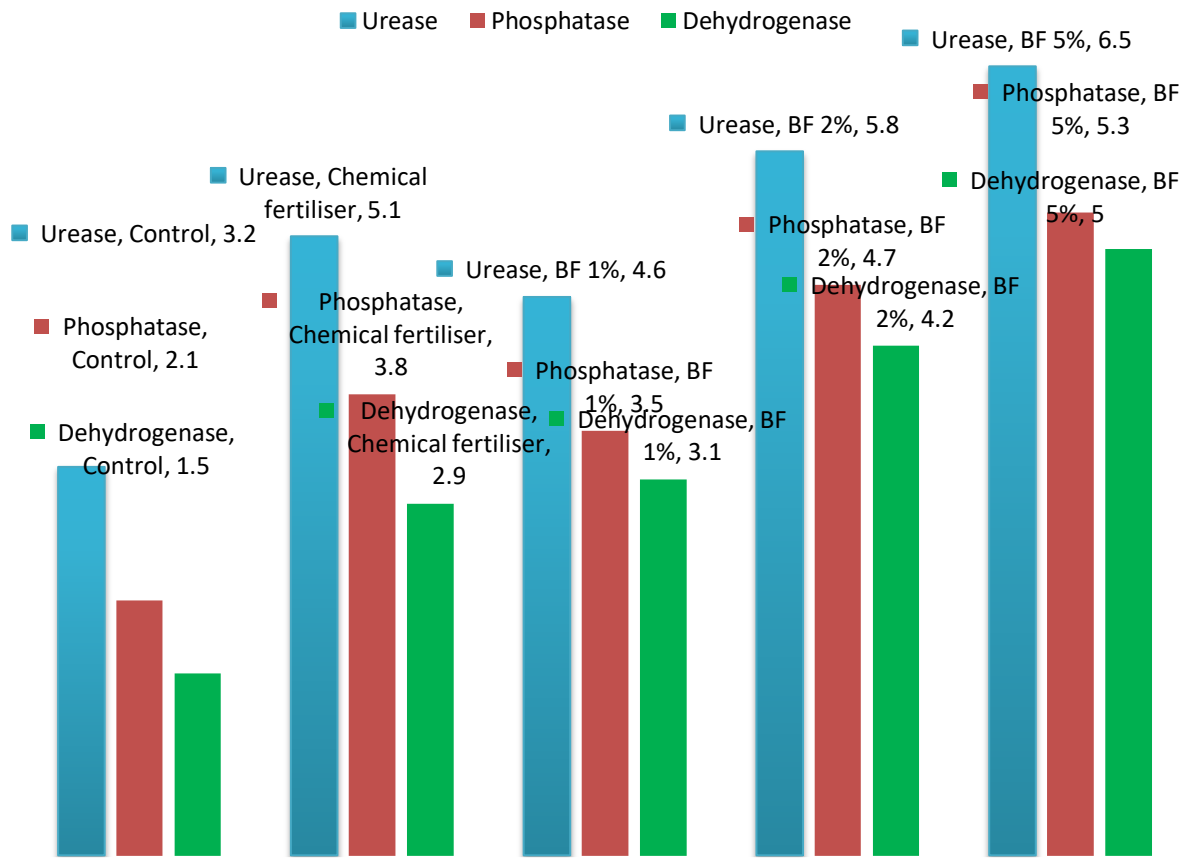

Figure 2: Germination Bioassay Results

Enzyme activities were significantly ($p < 0.05$) stimulated in all BF treatments as compared to control, with 5 % BF showing maximum values (103 % for urease, 152 % for phosphatase and 233 % for dehydrogenase more than control). Enzyme enhancement was significantly related to available N and P ($r > 0.85$); this indicated that microalgal organic matter was a stimulant to the soil microbial community.

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4 Plant growth promotion in pot trials

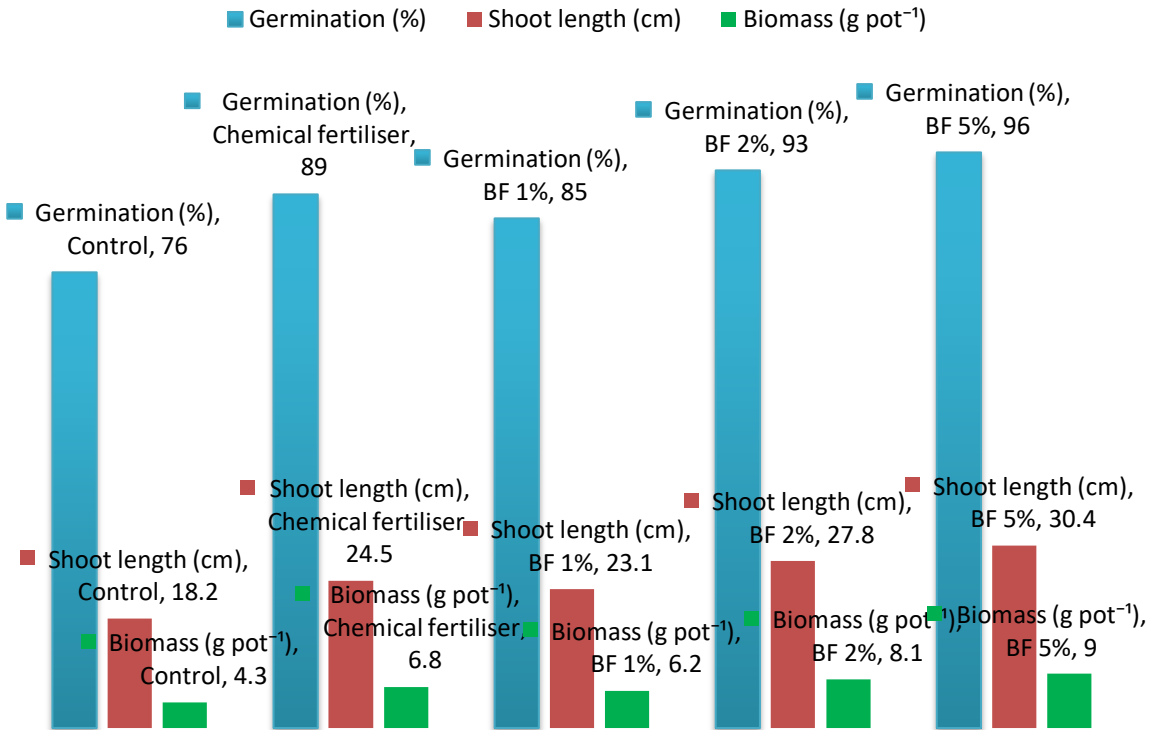

Figure 3: Plant Growth Promotion in Pot Trial

The 5% BF was superior to the chemical fertilizer under all three indicators, while the 2% BF was comparable **with chemical** fertilizer. The effect of the biostimulant (phytohormones) was clearly a positive intensification of the root/shoot growth apart from feeding.

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5 Soil microbial biomass and carbon status

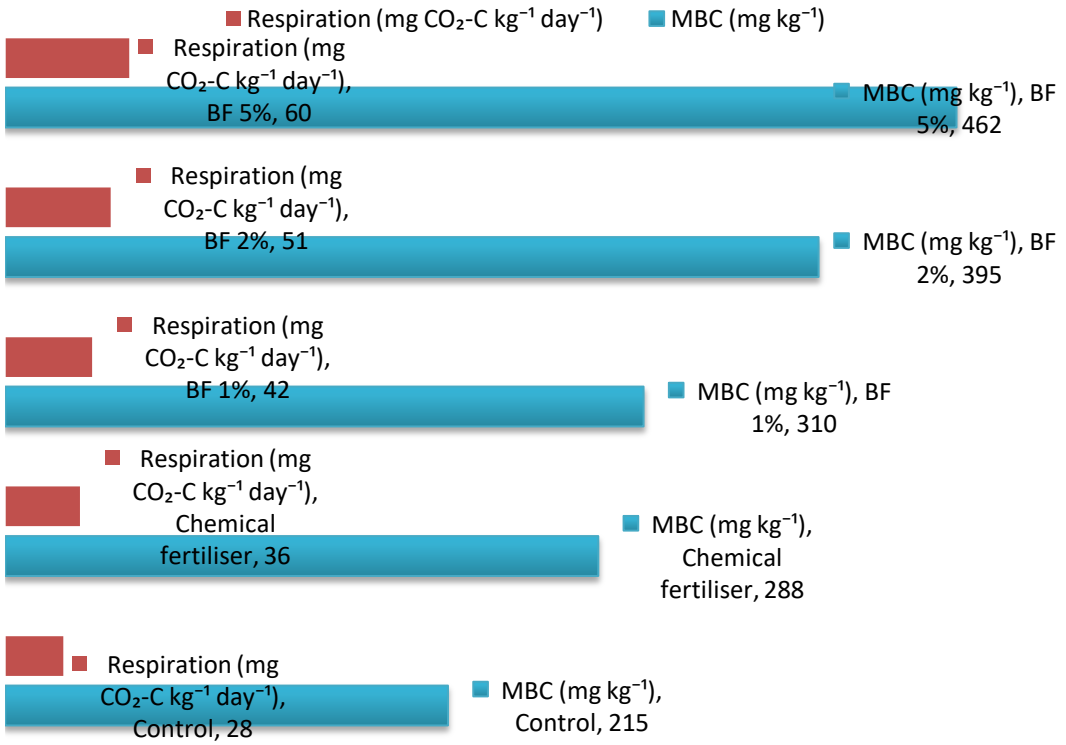

Figure 4: Soil Fertility Parameters Post-Harvest

BF treatments greatly improved MBC up to 115% above control and respiration, indicating a healthy microbial community. Dehydrogenase activity correlated significantly (r = 0.92) with the increase of MBC, indicating that the addition of microalgae has a positive effect on the biological fertility of soil.

5. Discussion

Microalgae can contribute to soil nutrient cycling and potentially improve plant growth due to its contribution to making nutrients more available and the production of bioactive substances like phytohormones [12]. Similarly, microalgae biomass and agrochemicals derived from it represent a special source of plant growth promoters that enhance the agriculture production and also provide resistance against disease [13]. Furthermore, microalgae enhance soil fertility and contribute to plant development and protection and provide an option to minimize our dependency on artificial fertilizers and pesticides [12]. The use of microalgae biomass, whose cultivation was performed on municipal wastewater, as biofertilizer improves the plant development in a similar manner to the other organic manures and conventional fertilizers but reduces nutrient losses to the soil [14]. The increased demand for food in this present world, where the use of chemical fertilizers pollutes the environment to a great extent, is the reason for adopting the biofertilizer practice on farms. Current advances in the biotechnology of microalgae related to the production of biofertilizers are described in this aspect, focusing on some of the ongoing research in this field and future directions for research and development. Moreover, the combination of wastewater treatment-based microalgae and the utilization of the resulting biomass as biofertilizers ... have been strongly emphasized for their application in the agricultural field[11]. As of now, we provide a circular economy approach with microalgae-mediated bioremediation and biorefinery strategies for the production of high-value metabolites as well as biofertilizer applications [13]. In the end, it is essential to keep agricultural productivity by adopting improved soil fertility management for food security challenges [15].

6. Conclusion

This study deals with basic and practical aspects of microalgal features that are relevant to key agricultural needs such as biological nitrogen fixation, soil phosphorus cycling, influence on soil microorganisms, plant growth promotion either via phytohormones or root association or cycling soil nutrients, biocontrol and soil stabilization. Furthermore, microalgae boost soil fertility and help with plant development and protection and provide an option to minimize our dependency on artificial fertilizers and pesticides. The use of microalgae biomass treated with municipal

wastewater as a biofertilizer positively affects plant development, as do other organic manures and conventional fertilizers and decreases nutrient loss to soil. Biofertilizers have become an essential need of modern farming systems due to growing demand to produce food to satisfy a world with a population of more than 2 billion people without polluting the environment with excessive application of synthetic fertilizers. In response to this challenge, we propose a circular economy approach to our value chain with microalgae-mediated bioremediation along with a biorefinery strategy for the production of high-value bioactive metabolites and as an enabling technology for biofertilizer. Finally, an important aspect of the food security issue is to be able to maintain productivity through improved soil fertility management.

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