Invited lecture/Review

Uncovering algae biomass potentials: from wastewater to biostimulants

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Abstract:
The need to minimize human impact on the environment encourages alternative methods and solutions which could provide new steps towards green transition. Population is growing and climate is changing which is causing distress in food production and to recycle resources is getting ever more important. It is already known that municipal wastewater is a nutrient-rich medium, furthermore there is great potential to implement new technologies for nutrient recovery. We must provide sustainable wastewater treatment and at the same time we must try to maximize utilization of all available resources found in wastewater. Wastewater is suitable for growing algal biomass which can later be used as biostimulants in crop production which is in line with the goals of circular economy. This article provides a short overview of several studies on algal wastewater treatment and types of biostimulants produced from algae biomass. Further research on algal biostimulants production using municipal wastewater need to be focused to pilot experiments and real wastewater since the majority of reviewed studies are done on the lab scale with synthetic wastewater.

Keywords: Nature-based solutions; High-rate algae pond; Agriculture; Reuse; Phytohormones.

1. Introduction

Growing population is causing global warming, extreme weather events and environmental changes, therefore striving to the green transition is essential, including environmentally friendly technologies, nature-based solutions and reuse of resources (Kapoore et al., 2021). Because resources are scarce, sustainable solutions to improve agricultural technologies and effective strategies for plant production are needed for providing the outcome without the negative consequences within production. Pressure on global food systems will be increasing also due to pollution caused by agriculture which is critical and causing ecosystem problems. Therefore, focus should be on finding new technologies and solutions for problems in agriculture that we are and will be facing (Calabi-Floody et al., 2018; La Bella et al., 2021; Shahzad et al., 2021).

In recent decades, algal biomass is gaining attention as it is versatile and can be used in a variety of industries, including wastewater treatment (Razaviarani et al., 2023). The use of algae in wastewater treatment was pioneered less than a century ago and have since been established worldwide (Oswald et al., 1957; Ho & Goethals, 2020). Process is mainly based on the biodegradation of organic matter and pollutants, driven by bacterial consortia and the implementation of microalgae as part of wastewater treatment depends on their ability to consume the organic or inorganic carbon, inorganic nitrogen and phosphorus in the wastewater for their growth, reducing concentration of certain substances in the water (Mohsenpour et al., 2021).

Microalgae (**Figure 1**) have shown great potential to grow in various types of wastewaters (Mohsenpour et al., 2021), moreover they have shown a high potential to remove contaminants from industrial waste or municipal sewage (Abdelfattah et al., 2023). High-rate algae ponds (HRAPs) contribute to the resource recovery, represent an alternative to conventional wastewater treatments and have great potential for the municipal wastewater treatment in locations with enough exposure to the solar radiation (Mehrabadi et al., 2017; Škufca et al., 2021). According to recent study by Kohlheb et al. (2020), HRAP is beneficial both environmentally and economically, contributing especially to CO_2 sequestration and reduction of eutrophication. There is also the potential of wastewater treatment using HRAP for production of low-cost biofuel (Mehrabadi et al., 2017). However, the performance of algal wastewater treatment is a remaining challenge due to variations in parameters like algal culture, wastewater composition, photobioreactor types, temperature and light intensity. Additionally, lack of an efficient method of harvesting microalgae from the culture medium has been a well-known problem (Zenouzi et al., 2013; Chawla et al., 2020).

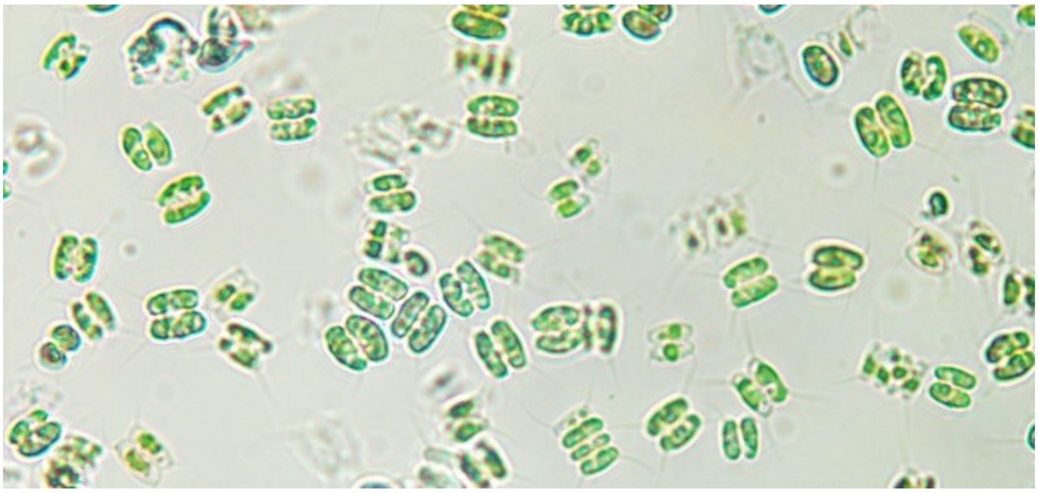

Figure 1. *Scenedesmus* sp. under a microscope (400x).

It is known that phytohormones, obtained from the diverse algae and cyanobacteria, may be applied commercially in agricultural land to enhance the crop productivity (Singh et al., 2017). Phytohormones are one of the biostimulants that can be produced by algae (Kapoore et al., 2021). Biostimulants can enhance the growth and quality of crops, improve mineral nutrient uptake and increase plant tolerance to abiotic stresses (Navarro-Lopez et al., 2020; Gonzalez-Perez et al., 2022). It is also important, that they could be applied in small quantities (Sharma et al., 2014). It is known that both, micro- and macroalgae, have biostimulant potential to support plant development and growth along with improved tolerance to abiotic stresses, but microalgae cultivation and harvesting is more difficult (Michalak et al., 2017) and have been explored less in the case of agricultural applications (Craigie, 2011). Because biostimulants have similar regulatory roles in microalgae as in higher plants (Lu & Xu, 2015), the application of microalgal specific components as biostimulants are gaining wide attention (Kapoore et al. 2021). Biostimulants represent sustainable alternative to synthetic plant protection products, are environmentally friendly, safe, and respect the human health, not generating chemical residues (Povero et al., 2016; Du Jardin et al., 2015). Therefore, they play a key role in sustainable intensification through efficiency of nutrients uptake from plants, enhancing productivity, yield and health of crops (Gonzalez-Perez et al., 2022). However, a major challenge remains in unravelling the mode of biostimulant action of specific bioactives due to variability of algal and crop species and their interactions, which depend on abiotic factors (Bulgari et al., 2019; Kapoore et al., 2021).

In this paper, we analysed the diversity of biostimulants from microalgal biomass that are relevant to agriculture and production technologies.

2. Methods

The literature review was carried out in the November 2023 using the Google Scholar (GS), Scopus and Web of Science (WOS) databases. Research through GS using search terms ’’microalgae, biostimulants, agriculture, wastewater’’ provided with more than 600 review articles, therefore more combinations of terms were used to narrow down the results. We searched through Scopus and WOS using keywords microalga* AND wastewater* AND biostimulant*, resulting 44 in Scopus and 32 searches in WOS. Without ’’*’’, there were only 26 results in both databases. We added keyword ’’agriculture’’, resulting in 15 hits in WOS (and 14 in Scopus). Search results were divided in two groups – review articles and primary research articles and thoroughly reviewed to determine if their content is relevant to the topic of algal biomass from wastewater and biostimulants.

3. Results and discussion

3.1. Biostimulatory compounds from algae biomass

Various bioactive components, including phytohormones, betaines, oligosaccharides elicitors, and microalgal protein hydrolysates, can be recovered from microalgae for production of plants. They could also contain amino acids, humic acids, fulvic acids, polysaccharides, antioxidants, vitamins, enzymes and others (Oancea et al., 2013; Kapoore et al., 2021). Some major groups of bioactive components are presented in **Table 1**. For example, in *Chlamydomonas* sp. and *Chlorella* sp. phytohormones like Gibberellic acid (GA) and ethylene (ET) play roles in growth, senescence, and other biological activities, therefore suggesting their potential commercial application in agriculture (Yordanova et al., 2010; Park et al., 2013; Tate et al., 2013).

Table 1. Bioactive molecules with stimulatory effects, found in algae.

Biostimulants and their effects		Examples	Growth media	References
Auxins*	Initiate cell elongation and root formation, provide stress tolerance (drough, salinity, heat) (Kapoore et al., 2021).	1. *Chlorococcum* sp., *Micractinium pusillum*, *Scenedesmus* sp., *Chlorella* sp. 2. *Chlorella vulgaris*	1. Tris-Acetate- Phosphate (TAP) medium. 2. Bristol Liquid Media.	1.Rupawalla et al., 2022. 2. Stirk et al., 2013.
Cytokinins*	Control cell division, fruit and flower development, stress tolerance (drought and heat…) (Kapoore et al., 2021).	1. *Chlorococcum* sp., *Micractinium pusillum*, *Scenedesmus* sp., *Chlorella* sp. 2. *Chlorella vulgaris*	1. Tris-Acetate- Phosphate (TAP) medium. 2. Bristol Liquid Media.	1.Rupawalla et al., 2022. 2. Stirk et al., 2013.
Gibberellins*	Initiate stem elongation, seed germination, flowering (Kapoore et al., 2021).	1. *Chlorococcum* sp., *Micractinium pusillum*, *Scenedesmus* sp., *Chlorella* sp.	1. Tris-Acetate- Phosphate (TAP) medium.	1.Rupawalla et al., 2022.
Abscisic acid*	Associated with stomatal closure, shoots growth inhibition, seed dormancy (Kapoore et al. 2021).	1. *Chlorococcum* sp., *Micractinium pusillum*, *Scenedesmus* sp., *Chlorella* sp.	1. Tris-Acetate- Phosphate (TAP) medium.	1. Rupawalla et al., 2022.
Proteins and amino acids	Major category for example: regulating nutrient uptake and enzymes, improve plant growth, metabolic signaling (Kapoore et al., 2021).	3. *Chlorella vulgaris* 4. *Spirulina* sp.	3. Sea or fresh water. 4. Fresh water.	3 and 4. Christaki et al., 2011.
Polysaccharides	Plant metabolic pathways, overall crop improvements, biotic and abiotic stresses protection (Rachidi et al., 2020; Kapoore et al., 2021).	5. 16 different species of mariculture microalgae	5. Medium t'2, medium fE containing EDTA or medium G2.	5. Brown, 1991.
Antioxidants, pigments, micronutrients	Various compounds, including some vitamins, chlorophylls and phenolics, often secondary metabolites (Kapoore et al., 2021).	6. 57 different strains (phylum *Chlorophyta* and *Cyanophyta*). 7. Two microalgal consortia made up of different strains (phylum *Chlorophyta* and *Cyanophyta*).	6. Coastal waters of western India. 7. Modified Bold's and Basal Medium, sewage wastewater.	6. Paliwal C et al., 2016. 7. Renuka N et al., 2017.
Auxin- and cytokinin like activity	Promote plant growth, improvements in germination and root formation (Navarro-Lopez et al., 2023; Renuka et al., 2018).	8. *Chlorella* sp., *Scenedesmus quadricauda*, *Coenochloris* sp., *Chlorosarcina* sp., *Tetracystis* sp., *Chlamydomonas* spp. 9. *Scenedesmus* sp. 10. *Scenedesmus obliquus*	8. Tamiya. 9. Synthetic treated wastewater. 10. Brewery wastewater.	8. Stirk WA et al., 2002. 9. Navarro-Lopez E et al., 2023. 10. Navarro-Lopez E, et al., 2020.

*phytohormones

3.2. Wastewater as medium for biostimulants production

High production costs of bioactives from algal biomass is a major obstacle for wider application, and wastewater as a nutrient-rich environment represents low-cost possibility for reuse of resources and fits circular economy definition (Kapoore et al., 2021). Currently, despite a high interest in developing natural biostimulants from microalgae, there is a lack of commercially available products, therefore it is crucial to identifiy strains with capacity to grow in wastewater and are highly productive (Morillas-Espana et al., 2022). Most of the studies are laboratory-based and should be taken on a pilot scale. Microalgae, such as *Scenedesmus obliquus*, grown in brewery wastewater, have the potential to act as plant biostimulants, contributing to sustainable and healthy food production in addition to wastewater treatment (Navarro-Lopez et al., 2020). *Scenedesmus* sp. was recently grown in simulated treated wastewater to obtain biostimulants, where cytokinin and auxin-like activity were found, improvements in germination and root formation appeared (Navarro-Lopez et al., 2023). Furthermore, Alvarez-Gonzalez et al. (2023), proposed wastewater as a suitable medium for growing microalgae strains *Synechocystis*, *Phormidium* and *Scenedesmus*, which can produce biostimulants in form of phytohormones. Cultivating microalgae in urban wastewater is offering new possibilities to identify and quantify the production of biostimulants, along with decreasing production costs of these high-value compounds (Alvarez-Gonzalez et al., 2023). Overall applications of algae biostimulants represent a promising approach in agriculture to reduce or substitute use of harmful chemicals when maintaining plant productivity (Ferreira et al., 2021; Dagnaisser LS et al., 2022).

Although studies have shown that algae are efficient in wastewater treatment, the compassion of effect of algal culture is still difficult due to several practical and economic challenges (Chawla et al., 2020; Mohsenpour SF et al., 2021). According to Kapoore et al., 2021, the major problem is risk of contamination with heavy metals, chemicals, self-care products and pharmaceuticals. There is a major hole of knowledge about pathogens and processes associated with transferring genes (Li et al., 2022). There are also difficulties with maintaining monoculture due to species or bacterial contamination and variety of seasonal and nutrient levels that are changing growth profiles. All these factors can highly affect composition of the end product of algae biomass (Kapoore et al., 2021).

4. Conclusions

Microalgae contribute to sustainability in crop production and wastewater treatment, stating the importance of integrating new technologies into environmental and agricultural practices. Production of biostimulants with algae-based technologies have big potential to achieve sustainable agriculture. We believe that it is possible to define methods to produce abundance of microbial biostimulants by growing and harvesting algae in municipal wastewater under controlled environmental conditions. The right balance between temperature, nutrient levels, water quality and other variables impacting successful microalgae growth and production of bioactive will be therefore the focus of our future research.

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Conflicts of Interest: The authors declare no conflict of interest.

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