

Utilization of Residues from Microalgal Industries for Agricultural Practices

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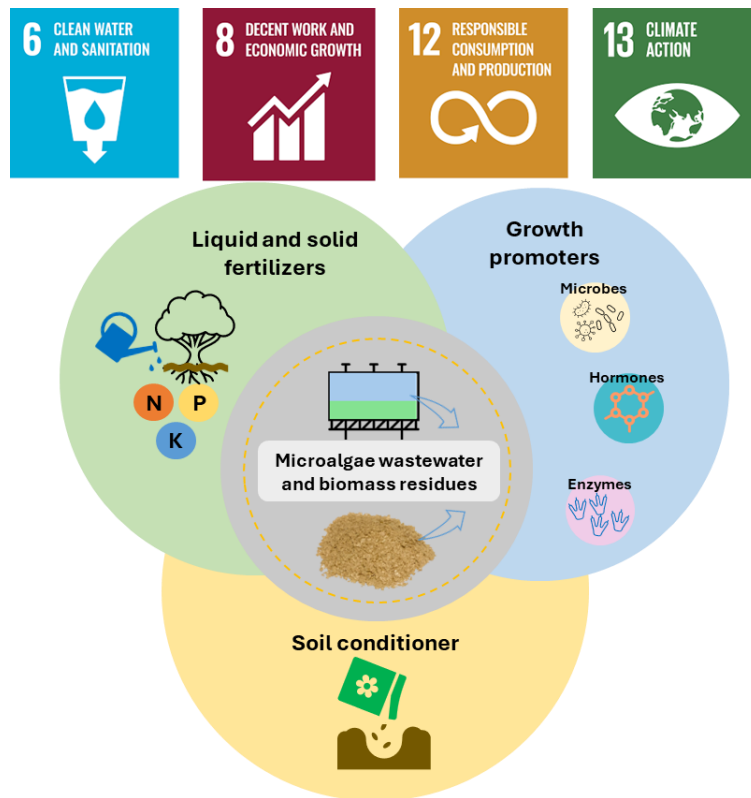
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Graphical abstract



Highlights

- Microalgae-derived waste biomass and wastewater promote sustainable farming practices
- Reutilization of microalgal processing wastewater as liquid fertilizer is feasible
- Microalgae biomass residues as biofertilizer and soil conditioner assist plant growth
- Resource recovery in microalgal industries benefit for circular economy

Abstract

Global consumptions of fertilizers and freshwater have been continuously growing due to increased food demand, leading to great concerns on food security. Bio-based resilient nutrient resources such as microalgae-derived waste biomass and wastewater have gained great attention as alternative solid and liquid fertilizer resources because they contain key nutrients (nitrogen and phosphorus), organic compounds (acting as soil conditioner, growth stimulators), and micronutrients. Thus, microalgae-derived solid and liquid fertilizers have great potential in promoting plant growth in soil and/or hydroponic farming, controlling release of nutrients to avoid nutrient leaching and volatilization, and facilitating to achieve circular economy in microalgal industries. However, several challenges, such as imbalanced nutrient element ratios, causes of heavy metal accumulation and increased pH/conductivity, may limit their wide applications. Several recent-published review articles have documented the application of fresh microalgal biomass as fertilizer sources via direct use and conversion methods or recycling cultivation medium for microalgal growth, but no review has been conducted on utilization of microalgal processing wastewater and biomass residues for agriculture practices. Herein, this article provides a comprehensive review on the processes relating to recovery of resources (water, nutrients, valuable plant growth compounds) from microalgae processing wastewater and biomass residues generated in microalgal biorefinery industries, and identifies the key factors that are associated with the resource recovery efficiency and their effects on plant growth.

Keywords: Biofertilizers; Biomass residues; Liquid fertilizer; Nutrient recovery; Soil conditioner; Water reuse

Outline

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1. Introduction

Climate change is impacting on global socio-economic development as it is relevant to cause disasters such as gradual increasing in temperature, changing in terrestrial and aquatic ecosystems, melting glaciers, rising sea levels, and disrupting rainfall patterns [1-3]. Accordingly, the changes in hydrological cycle and microbial compositions/enzymatic activities in soil unexpectedly occurred, leading to negative impacts on habitat, plant productivity, and whole food chain [4-7]. Greenhouse gases (GHGs) emission has been recognized as one of the main contributors to global climate change, which is majorly linked to human activities such as industrialization, energy consumption, transportation, and land use (i.e., reduction of agricultural and forestry land) [8, 9]. Among the major GHGs, carbon dioxide (CO₂) emission contributes to ~80% of the total GHGs emission (6343.2 million metric tons of CO₂ equivalent), as reported by the US Environmental Protection Agency [10].

To combat GHG-induced climate change, utilization of microalgal biomass for simultaneous fixing CO₂ from atmosphere and producing commercial high value-added products (such as lipids, proteins, carbohydrates, fats, vitamins, pharmaceuticals, food supplements) has been adopted as one of feasible solutions and has been well commercialized [11, 12]. It has been documented that microalgae can capture ~1.83 kg of CO₂ per kg biomass; and photosynthesis-based CO₂ reduction is estimated almost 100 gigatons annually, which facilitating achieving the goal of Paris Agreement, i.e., limiting global temperature increase below 2°C [13-15]. During microalgal cultivation under well-controlled conditions (pH, temperature, salinity, light intensity, nutrient level), they display their unique metabolic pathways for accumulating certain valuable components, which can be extracted and purified by proper biorefinery approaches and converted into commercial bioproducts [16, 17].

However, microalgal biorefinery industries suffer challenges during microalgal cultivation and harvesting (i.e., concentrating the culture solution towards higher microalgal solid contents for downstream processing), as well as purification of target products [18, 19]. For example, the cultivation of microalgae requires intensive water and nutrients (i.e., cultivation medium for promoting microalgal growth), and energy consumption, significantly raising the overall cost of microalgae-based products [20, 21]. After microalgal growth, the spent cultivation medium was separated from microalgal cells and considered as wastewater (hereinafter named as microalgal processing wastewater); meanwhile, after extraction of valuable products from microalgal cells, wet/dried residual biomass wastes are produced. Both solid and liquid wastes contain certain amounts of carbohydrates, proteins, lipids, nutrients, minerals, etc., although their quantity and quality are strongly associated with microalgal species, cultivation conditions, and valuable product components [22]. Towards achieving a circular economy, there is an increasing interest in recovering resources from such wastes generated in microalgal industries, especially for agricultural practices (Figure 1). In 2023, the global microalgae market was estimated at ~4.01 billion USD and it is predicted to increase to ~7.31 billion USD by 2032 with the compound annual growth rate of 6.9%. This highlights the favorable market trend for the large-scale implementation of resource recovery technologies into microalgal biorefinery industries [23, 24].

It shall be noted that microalgae biomass production and its valorization into biofuel, biofertilizers and other valuable products have been well reviewed [25-28]. The advances in technological developments to improve microalgal production and their applications as biofertilizers have been comprehensively discussed [29-31]. To our best knowledge, utilization of microalgal biomass-derived waste and microalgal processing wastewater as fertilizers for agricultural practices has not been comprehensively reviewed. In this study, we aim to review the most recently published

studies on resource recovery from microalgal-derived waste and wastewater for agricultural sector under the circular bioeconomy framework. The technical advances in converting both solid and liquid wastes to valuable resources will be summarized and the effects of process operation parameters on resource recovery efficiency and plant utilization potential will be discussed.

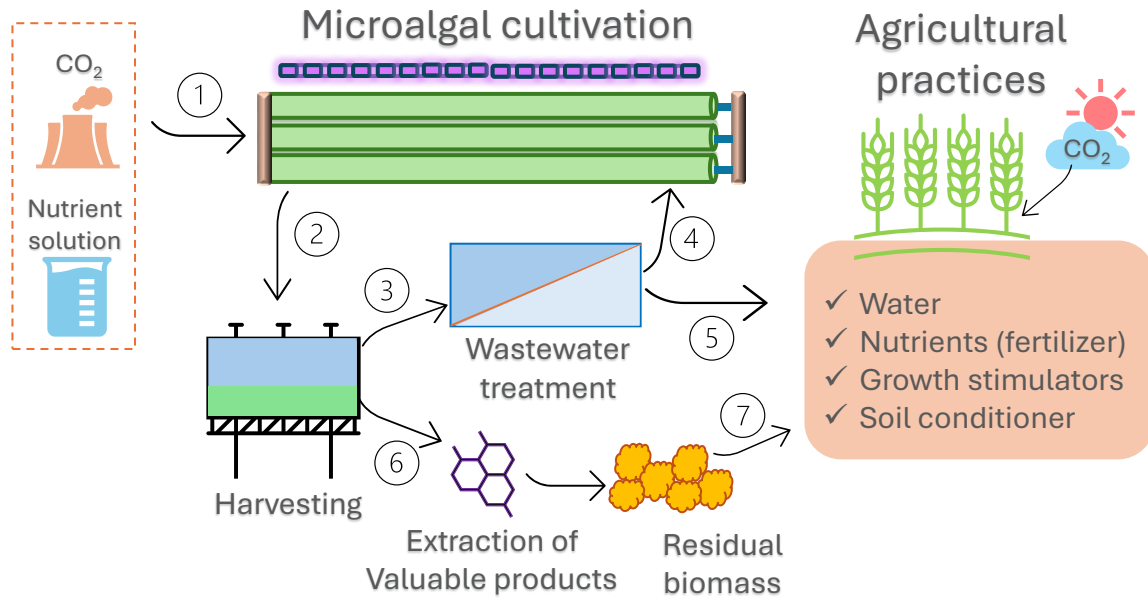


Figure 1. Resource recovery from microalgal processing wastewater and residual biomass waste for agricultural practices (1. Microalgal cells use CO₂ and nutrient solution for growth; 2. Microalgal cells are separated from spent cultivation solution in a microalgal harvesting process; 3. The spent cultivation solution is considered as the processing wastewater for treatment; 4. Treated water can be recycled for microalgal growth; 5. Treated water can be used for agricultural practices; 6. Harvested microalgal cells are used for extraction of valuable products; 7. After extraction, residual biomass can be used for agricultural practices).

2. Utilizing microalgae-derived wastewater for agricultural practices

2.1 Microalgal processing wastewater treatment towards recycling

Microalgal cultivation for biomass production is a water- and nutrient-intensive process, e.g., >1 m³ of fresh water, along with 10-150 g nitrogen and 7-23 g phosphorus per kg of dried microalgal

biomass considering diverse cultivation systems (such as photoreactors, open pond, etc.) and microalgal species [32, 33]. The direct cost of microalgae cultivation medium is estimated at 1.17-49.62 USD/m³, leading to 4.64-301.61 USD/kg dried biomass [34]. While, after microalgal cultivation and harvesting, more than 80% of cultivation solution is finally treated as wastewater for discharge, which contains microalgae cell debris (including solids and organics), residual nutrients, and minerals [35]. In some regions, using freshwater to dilute the cultivation wastewater to meet discharge standard. However, this approach would increase the water footprint for microalgal production and cause environmental concerns, especially marine ecotoxicity based on the life cycle assessment analysis [32, 36]. Therefore, recovering water or/and nutrients from the microalgal processing wastewater is adopted, which aids in improving the economic and environmental feasibility of microalgae production for large scale commercial applications [37].

In terms of microalgae processing wastewater, diverse techniques have been reported for its treatment towards reuse purposes. It is noted that the conventional processes (such as coagulation, flocculation, adsorption) require additional capital cost for chemicals/adsorbents, and the treated water quality may not well meet the wastewater reuse standards. Among the techniques for simultaneous separation of water and nutrients from unfavorable components (microalgal cell debris, bacteria, toxic organics, unassimilated ions, etc.) in the wastewater, membrane-based processes have been considered as a techno-economic feasible method, especially in microalgae cultivation systems used for extraction of high-quality food-grade products. A recent study further addressed that membrane-based microalgal reutilization process could achieve less global warming potential, especially when the membranes were made of the recycled waste materials [38, 39].

It shall be realized that membrane processes can also be adopted for microalgal harvesting, which have been well documented in many review articles [40-44]. While, both microalgal cultivation solution (for harvesting) and the microalgal processing wastewater (after harvesting) display different compositions in terms of solids, organics, ions, therefore, dissimilar membrane materials and processes, operation conditions, and fouling control strategies are expected. Here, we focus on the membrane-based treatment of microalgae wastewater effluent after harvesting process towards its recycling application (Figure 1).

In the previously reported literatures (Table 1), pressure-driven membrane processes, including microfiltration and ultrafiltration are mainly employed for microalgal wastewater treatment, considering cost and energy consumption, commercial availability, and operation feasibility. Under hydraulic pressure-driven (i.e., using permeate pump) or gravity-driven (i.e., water head) conditions, microfiltration (0.1 to 10 μm) and ultrafiltration (0.002 to 0.1 μm) membranes were applied to reject large-sized suspended solids such as colloids and microalgal cells, meantime allowing the smaller-sized substances (such organics, nutrients, ions) to pass through them (Figure 2). For example, Kristjánsdóttir et al. [45] employed gravity-driven and crossflow microfiltration and ultrafiltration processes for recycling microalgae processing wastewater, and found that the treated water met the agricultural reuse requirements. In addition, it was found that the cake fouling mechanism was independent with wastewater properties, membrane type, and filtration condition. With periodic water flushing, such cake fouling could be effectively alleviated.

In some scenarios, the MF/UF permeate water may still contain small-sized organic compounds that are produced by microalgae during their cultivation, or metal elements that could not be fully utilized by microalgae. Among them, certain unfavorable components (such as humic acid, aromatic organics, Hg) would perform as growth inhibitors, which may negatively influence its

subsequent reuse in agricultural practices. Thus, additional adsorption (e.g., activated carbon), advanced oxidation processes (AOP), and electrolysis could be considered as potential post-treatment processes of membrane filtration (Table 1 and Figure 2) [39, 46, 47]. It is noted that adsorption processes require additional capital and operation costs for adsorbent materials and their regeneration units; AOP and electrolysis demand expensive facilities and high energy consumption. Whether such increased cost could be compromised by the benefits from improved plant growth has not well examined.

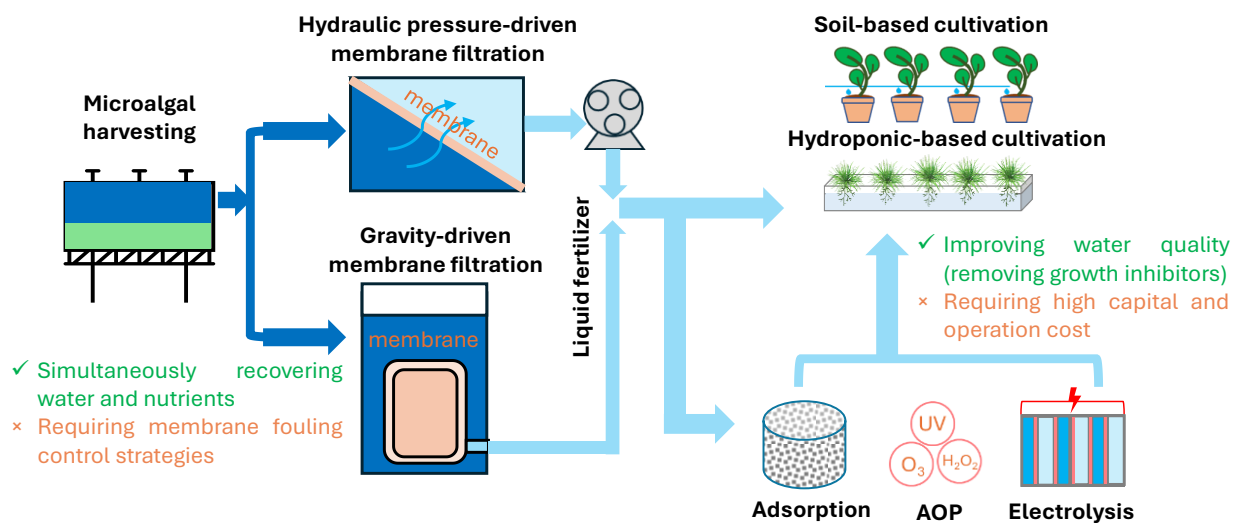


Figure 2. Schematic diagram to illustrate membrane-based processes in treating microalgal processing wastewater for plant cultivation

Nevertheless, research and practices on large-scale membrane processes for recycling microalgal processing wastewater as liquid fertilizers towards long-term sustainability are still limited [39, 48]. Considering different cultivation conditions for diverse microalgae species and target valuable compounds, the processing wastewater matrix could be dissimilar in terms of compositions and concentrations of nutrients and biostimulant compounds. Accordingly, the adopted membrane processes need to be carefully optimized in order to maximize nutrient recovery, improve

membrane performance, and reduce energy consumption. For example, membrane materials that have limited binding affinity with nutrients and biostimulant compounds, and membrane pore sizes that allow the recovered elements to pass through shall be selected. The membrane processes for microalgae processing wastewater require relatively high-energy input for membrane fouling control, representing the main bottleneck for their large-scale applications. Membrane fouling is generally caused by pore blocking and cake formation due to the presence of organic compounds (polysaccharides, proteins, lipids and bioactive molecules) in microalgal processing wastewater. Therefore, suitable low-energy physical membrane cleaning approaches (such as periodic ultrasonication and vibrating membranes) and modification of membranes/membrane modules to alleviate membrane fouling shall be considered with optimization of operation conditions, towards improving treatment efficiency [49-51]. The membrane performance and treated water quality, as well as their relationship with the subsequent reuse efficiency (such as plant growth profiles, health impacts of plants) also need to be comprehensively investigated to unlock potential technical and economic challenges for scaling-up.

2.2. Reuse practices of treated microalgal processing wastewater

Recycling water from microalgal processing wastewater for subsequent microalgal cultivation has been recognized as the widely applied reuse option, towards reducing freshwater usage in microalgal industries. However, the dissimilar findings could not lead to a conclusive approach to achieve positive microalgal growth with water recycling, which have been well discussed in recently published review articles [37, 52-54]. It appears that several key factors such as cultivation system, harvesting techniques, recycling mode and frequency, pre-treatment strategies, and microalgal species, could greatly influence microalgal growth patterns with the recycled water. In addition, potential challenges associating with its influence on quality and quantity of microalgal

biomass and their target products are raised, including (1) limited residual nutrients in the recycled water, therefore additional nutrients need to be dosed or the recycling ratio needs to be optimized towards achieving the desired microalgal growth rate [46]; (2) the presence of inhibitors (such as smaller-sized metabolic products of microalgal cells, accumulated metal ions) that limit microalgal growth and importantly influence excretion of target products, therefore selection of proper treatment approaches with effective mitigation of inhibitors should be adopted [37, 55].

Table 1. A summary of membrane-based treatment techniques for producing liquid fertilizer from microalgae processing wastewater

Microalgal strain	Membrane	Reuse purpose	Effect on growth	Ref.
<i>Arthrospira platensis</i>	Filter (20 μ m)	Microalgal cultivation (<i>A. platensis</i>)	Accumulation of organic matter in the recycled medium led to lower microalgal growth than that with fresh medium.	[56]
<i>Scenedesmus acuminatus</i>	UF (PVDF, 50 kDa)	Microalgal cultivation (<i>S. acuminatus</i>)	Hexadecanoic acid and octadecanoic acid in the recycled medium caused microalgal growth inhibition.	[55]
<i>Arthrospira platensis</i>	Filter (20 and 40 μ m) followed by adsorption	Microalgal cultivation (<i>A. platensis</i>)	Using 75% of treated medium with 25% fresh medium led to comparable biomass and protein production as 100% fresh medium.	[46]
<i>Spirulina maxima</i>	Filter (50 μ m) followed by adsorption	Microalgal cultivation (<i>S. maxima</i>)	The recycled medium led to comparable microalgal growth as the fresh medium.	[57]
<i>Scenedesmus acuminatus</i>	MF (PES, 0.2 μ m) or UF (100 Da) followed by adsorption	Microalgal cultivation (<i>S. acuminatus</i>)	Humic acid in the recycled medium inhibited microalgal growth.	[58]
<i>Spirulina</i>	MF followed by UV, or electrolysis, or electrolysis-UV	Microalgal cultivation (<i>Spirulina</i>)	The treated medium (after MF-electrolysis-UV) led to comparable microalgal growth as the fresh medium.	[39]

<i>Nannochloropsis gaditana</i>	MF (1.2 µm) followed by ozonation	Microalgal cultivation (<i>N. gaditana</i>)	The treated medium promoted microalgal growth and protein/liquids production.	[59]
<i>Dunaliella salina</i>	UF (PS, 100 kDa) followed by AOP	Microalgal cultivation (<i>D. salina</i>)	The treated medium led to comparable microalgal growth and carotenoids production as the fresh medium.	[60]
<i>Scenedesmus acuminatus</i> GT-2	UF (50 kDa) followed by AOP and adsorption	Microalgal cultivation (<i>S. acuminatus</i> GT-2)	Fulvic-like and humic-like matters in the treated water inhibited microalgal growth.	[47]
<i>Haematococcus pluvialis</i>	MF (PVDF, 0.2 µm) or UF (PS, 100 kDa)	Plant cultivation: basil (<i>Ocimum basilicum</i>) and lettuce (<i>Lactuca sativa</i>) in hydroponics	Both plants cultivated with the permeate grew slower than those with the commercial fertilizer.	[45]
<i>Scenedesmus Tetradesmus</i>	MF (PVDF, 0.03 µm)	Plant cultivation: zucchini (<i>Curcubita pepo</i> L.) in hydroponics	The root dry weight and stem diameter was lower than those with the standard medium.	[61]

AOP: advanced oxidization process; BOD: biological oxygen demand; MF: microfiltration; PES: polyethersulfone; PS: polysulfone; PVDF: polyvinylidene fluoride; TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus; TSS: total suspended solids; UF: ultrafiltration

As shown in Table 1, recently reported membrane-based treatment of microalgal processing wastewater mainly focus on its reuse in microalgal cultivation. Alternatively, simultaneous recovery of water and nutrients from microalgal processing wastewater as liquid fertilizers for plant cultivation have been attempted in a few studies [45, 62], offer a promising solution to conquer global water and fertilizer shortages in agricultural sectors [63, 64]. However, in some reports, the liquid fertilizer derived from microalgal processing wastewater did not compete with the commercial fertilizer in soil-based systems, possibly due to their insufficient nutrients (N, P, K), accumulation of salts, inadequate or excessive trace minerals, or an imbalanced nutrient/ion concentration. For example, researchers found the residual *Scenedesmus almeriensis* cultivation wastewater as irrigation water for *Pelargonium hortorum* could negatively impact the plant height

and diameter, with the optimal irrigation solution as the control [65, 66]. Thus, regulating nutrient compositions in the recycled water by blending it with fresh water or additional required nutrient elements is necessary. The salinity-induced negative impact was considered as another challenge, thus selection of salt-tolerant plants for this cultivation system could be an approach to improve liquid fertilizer reuse efficiency. It shall be noted that such studies did not compare plant growth in terms of freshwater consumption, which is also an important operation cost contributor for plant cultivation.

Recently, hydroponics or vertical farming has gained great global interest due to its advantages such as high crop yields, less use of land and water resource, and environmental-sustainable nature [67]. Major challenges that limit the widespread implementation of hydroponics include high costs for infrastructure and fertilizer supply [68, 69]. Henceforth, the treated microalgae processing wastewater has been considered as a promising liquid fertilizer source to operate hydroponic systems, although such attempts are limited in the documented literature. Morillas-España et al. [61] utilized the UF-treated microalgal processing wastewater to produce zucchini (*Curcubita pepo L.*) in the hydroponics. However, it was found that the plant root dry weight and the stem diameter with the treated water were lower than those with the standard fertilizer solution. Similarly, Kristjánisdóttir et al. [45] also found the lettuce and basil displayed slower growth profiles (in terms of leaf length and number, plant length and root length) in hydroponic systems fed with MF-treated *Haematococcus pluvialis* microalgal processing wastewater compared to those with the commercial fertilizer solution, even both irrigation solutions contained the similar nutrient levels. Such retarded growth profiles were possibly attributed by the insufficient amounts of certain essential microelements and higher levels of certain heavy metals in the recycled liquid fertilizer.

Although microalgal processing wastewater contains relatively limited toxic compounds, it may contain certain metal elements and metalloids (such as boron, cobalt, copper, iron, manganese, molybdenum, nickel, zinc, etc.) that are micronutrients for microalgal growth [70]. The currently reported membrane-based microalgal processing wastewater treatment processes could not fully remove these metal and metalloids, although such treated water could meet the national and international wastewater reuse standards. Due to the unique behaviors of plants in taking up metal and metalloids (such as root uptake, mobilization, cellular compartmentation, and sequestration) [71], their accumulations in plants irrigated with the recycled liquid fertilizers are expected, therefore raising great concerns on their public health impacts [72, 73]. Especially, this aspect needs to be carefully evaluated if the recycled liquid fertilizer is applied for agricultural irrigation of human consumed foods (such as edible leafy vegetables) [64]. Some studies have addressed this consideration by evaluating the heavy metal-determined health risk index (HRI, i.e., non-cancerous toxic potential during a lifetime period) and targeted hazard quotient (THQ, TQH <1 indicating almost no health effects) values. For example, a study highlights that the THQ values for all the heavy metals in hydroponic-cultivated basil and lettuce plants irrigated with treated microalgal processing wastewater was 0.79 and 1.58 respectively, which was comparable to that with the commercial fertilizer solution (0.81 for basil; 1.35 for lettuce). It appears that basil is a suitable vegetable for growing with the recycling liquid fertilizer considering its low potential adverse health effect on human consumption [45]. To conquer the potential effects of heavy metal elements in the treated water on plant growth, risk mitigation strategies such as blending the treated water with clean water, employing a pre-treatment process (such as chemical precipitation, adsorption), shall be implemented and evaluated. Importantly, the additional treatment processes could not compromise the recovery efficiencies of nutrients and biostimulant compounds.

In addition, plenty of research studies have revealed that microalgae is capable to produce diverse biostimulant molecules (e.g., auxins, cytokinins, betaines, amino acids, vitamins and polyamines) under favorably controlled conditions, which would be used to promote plant growth and alleviate biotic and abiotic stress (Figure 3) [74, 75]. For instances, polysaccharides that play a key role in initiating the synthesis of antioxidant enzymes (glutathione and xanthine oxidase) and are responsible for protecting plants from oxidative stress [76]; trace amount of phytohormones that could activate enzymatic and non-enzymatic antioxidant defense mechanisms in plants [77]. Most microalgae-based biostimulants are stored inside of cells and their extraction requires additional physical or chemical treatment of microalgal cells. On the other hand, during the microalgal cultivation processes, certain amount of biostimulants may also be released from the living microalgal cells and present in the used cultivation medium. As a result, such growth stimulators may benefit plant cultivation. Several studies have reported that the biostimulant activity of microalgal secreted phytohormones could improve the seed germination rate, plant growth and development by increasing the nutrient uptake, enhancing the photosynthetic activity, and improving the crop resistance against extreme environmental conditions, as summarized in Table 2.

For example, Alling et al. [78] compared the seed germination indexes of tomato (*Solanum lycopersicum*) and (*Hordeum vulgare*) cultivated with the filtered microalgal (*Chlorella vulgaris*) cultivation medium and broken microalgal biomass (i.e., containing biostimulants), and found their comparably higher germination indexes than the control (with distilled water). Çakmak and Uzuner [79] applied the residual cultivation media of microalga (*Chlorella vulgaris*) for irrigation of tea plants with achieving 23%-26% higher chlorophyll accumulation, 62%-69% longer node length, 5%-36% longer leaf length, and 23%-54% more leaf width. Kholssi et al. [80] noticed that

the membrane-filtrated *Chlorella sorokiniana* cultivation solution (BG11 as the liquid medium) contained residual nutrients and extracellular substances excreted by microalgae, which facilitated the *Triticum aestivum* seed germination and plant growth (increasing length by 30%, dry biomass of stem and leaves by 22%, roots by 51% after 15-day cultivation). It is noted that the detailed analysis of biostimulant compounds and their concentration levels in the recycled liquid fertilizer has not been well conducted yet, which needs to be further investigated in detail.

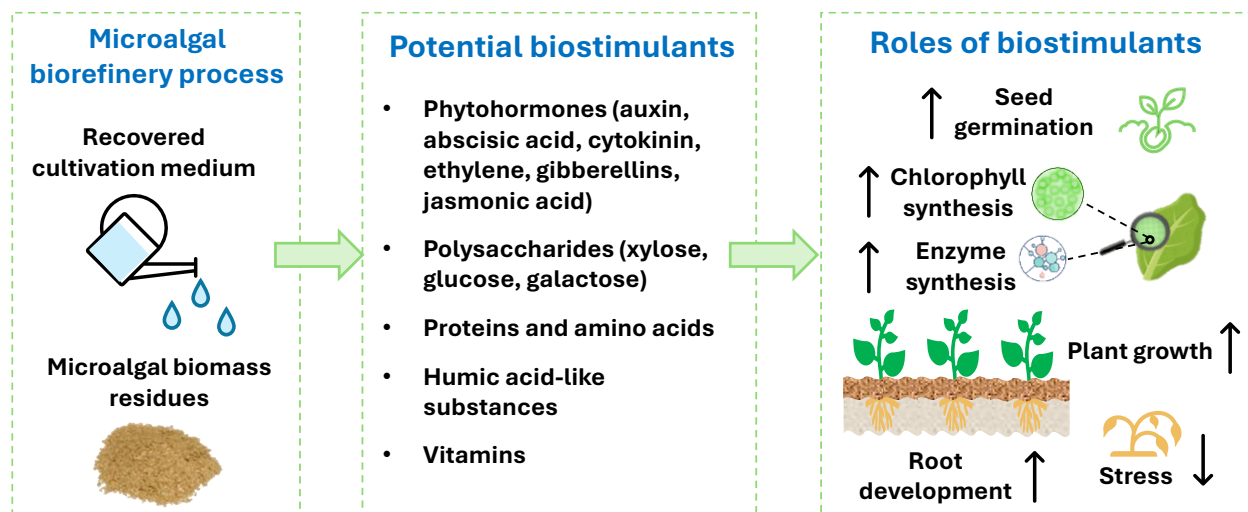


Figure 3. Potential biostimulants derived from recovered cultivation medium and microalgal biomass residues and their roles in promoting sustainable agriculture

Table 2. Application of microalgal wastewater/wastes as biostimulants and their potential positive effects (+) on plant growth

Microalgal wastewater/wastes	Testing plant	Seed germination	Stem development	Root growth	Chlorophyll contents	Ref.
<i>Scenedesmus</i> and <i>Tetradesmus</i> treated wastewater	<i>Lepidium sativum</i> L.	+	+	+	+	[61]
<i>Chlorella vulgaris</i> and <i>Scenedesmus obliquus</i> culture supernatant	<i>Solanum lycopersicum</i> and <i>Hordeum vulgare</i>	+				[78]
<i>Chlorella vulgaris</i> cell-free supernatant	<i>Camellia sinensis</i>		+		+	[79]

<i>Chlorella fusca</i> LEB 111 and <i>Spirulina</i> sp. LEB 18 culture supernatant	<i>Lycopersicon esculentum</i> seeds	+	+	+		[81]
Filtered <i>Chlorella sorokiniana</i> cultivation solution	<i>Triticum aestivum</i>	+	+	+	+	[80]

3. Utilizing microalgae-derived waste for agricultural practices

3.1 Direct utilization of microalgal wastes

After the extraction of valuable products from microalgal biomass, a surplus amount of biomass residues is generated and considered as solid waste, even though it still contains nitrogen, phosphorus, potassium, protein, carbohydrates and other essential micronutrients [82-84]. Several review articles well documented that the dried microalgal biomass wastes could be converted to bio-oil by employing energy-intensive strategies (such as thermal pyrolysis) [85, 86], and animal feeds by biorefinery processes [87, 88]. Alternatively, the microalgal biomass residues could be directly used as biofertilizers, biostimulants, or soil conditioners to improve soil fertility and crop production in agricultural sector (Figure 4), similarly as the behaviors of fresh microalgal biomass [89]. It has been well documented that the fresh microalgal biomass could (1) provide key nutrients (nitrogen, phosphorus, potassium, NPK; certain micronutrients) and growth promoter (e.g., phytohormones, including gibberellins, auxins and cytokinins) to facilitate plant development with tolerance to biotic and abiotic stress [90, 91]; (2) control release of nutrients into the soil and avoid the loss of nutrients by leaching and volatilization due to high porous nature of microalgae waste after extraction processes [92]; and (3) enhance root and soil microbial activities via nitrogen (N₂)-fixation and P-solubilization [93, 94].

It is anticipated that with the reuse of residual biomass for agricultural practices, a closed microalgae production cycle can be achieved without disposal of solid waste into the environment. Although the presence of NPK in the microalgae biomass residues allowed them to be considered as an alternative for chemical fertilizers, the NPK levels were associated with the microalgae species, compositions of microalgae cultivation solution, and extraction conditions (such as temperature, pressures, use of chemicals) [84]. Some research work has addressed that direct use of microalgae waste (e.g., *Chlorella vulgaris* after sonication) in soil could benefit for significant increase of plant growth in terms of node length, leaf width, or leaf length [95]. While other studies found microalgae waste (e.g., *Chlorella vulgaris* after extraction of oil) only promoted the root development and production of carotenoids and chlorophyll contents in plants (lettuce), instead of promoting plant leave number/length, and wet/dry weight [96]. Further analysis of rhizosphere microbial community revealed that the direct use of microalgae waste tended to cause the reduced relative abundances of certain groups of microorganisms (*Ascomycota* and *Basidiomycota*) that could help nutrient absorption and enhance soil quality, therefore leading to lower lettuce production. Such dissimilar observations may be associated with the different cultivation environments and soil types, microalgae and plant species, waste biomass treatment methods, etc. In particular, the different patterns of nutrient availability and rhizosphere microbial community structures in soil would play key roles in inducing dissimilar protein and chlorophyll synthesis in plants.

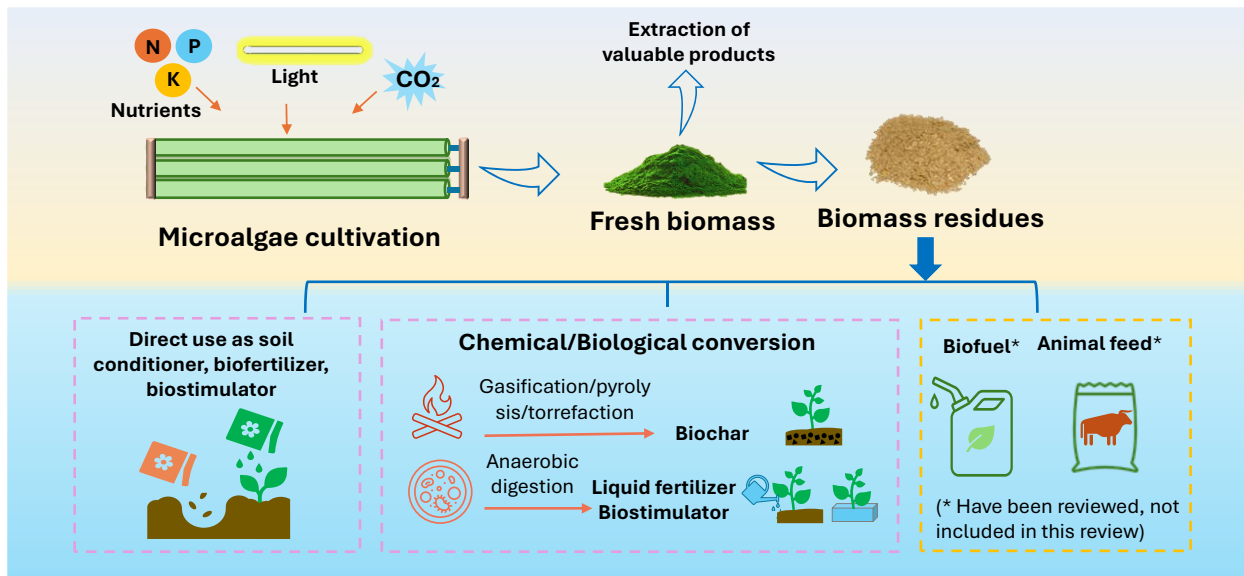


Figure 4. Application of microalgae biomass residues for agricultural practices.

It has been documented that residual microalgal biomass plays a key role in enhancing rhizosphere management (e.g., facilitating certain microbial community development), leading to improved plant growth and production [97], as summarized in Table 3. It is noted that continuous plant cultivation destroys the microbial community structure and causes the depletion of organic carbon and nutrients in soil, subsequently resulting in poor plant growth with reduced protein content. Moreover, the presence of phenolic compounds in soil may induce the loss of anti-pathogenic microbial species with extending cultivation period. While the use of bio-based fertilizers tends to enhance microbial activity to improve soil fertility and nutrient bioavailability, e.g., increasing the abundance of nitrogen-fixing and nutrient-cycling taxa and fungal community within the soil [98]. The biomass residues also play key roles as biostimulants by providing growth-promoting substances like auxins, indole acetic acid, and gibberellic acid, which accelerates plant growth (Figure 3), although such biostimulants have not well qualified and quantified in current-reported studies.

Table 3. Roles of microalgal biomass residues in influencing soil property and plant production

Microalgae biomass residue	Tested plant	Influence on soil property	Influence on plant growth	Ref.
<i>Chlorella pyrenoidosa</i> biomass after oil extraction	<i>Lactuca sativa</i>	Increasing abundance of nitrogen-fixing and nutrient-cycling taxa; increasing decomposition of soil organic matter and nutrient release	Promoting protein and chlorophyll synthesis	[98]
<i>Tetradesmus obliquus</i> biomass after biostimulant extraction	<i>Vigna radiata</i> L. <i>Cucumis sativus</i> L. <i>Lepidium sativum</i> L.	Increasing organic carbon, phosphorus, and Zn contents as well as C/N ratio; increasing removals of hydrocarbons, heavy metals, trace elements from soil due to the presence of biosurfactant and bioemulsifying fractions	Improving germination and formation of roots	[84]
<i>Scenedesmus</i> sp biomass after oil extraction	<i>Oryza sativa</i> L.	Increasing bioavailability of N, P and K; increasing decomposition of organic matter	Increasing protein and amylose content	[99]
<i>Nannochloropsis salina</i> biomass after lipid extraction	<i>Panicum coloratum</i> L.	Increasing organic carbon and nitrogen mineralization	Increasing plant growth and development	[100]
<i>Chlorella pyrenoidosa</i> biomass after oil extraction	<i>Lactuca sativa</i> L.	Increasing bioavailability of nutrients	Enhancing enzymes activity for chlorophyll biosynthesis	[96]

Under certain conditions, only application of microalgae biomass residues as fertilizers may not be sufficient or not be present in an optimal element ratio of NPK. Thus, the microalgae biomass residues are generally combined with commercial inorganic fertilizer to maximize plant growth. For example, Nayak et al. [99] combined the microalgae biomass waste (*Scenedesmus* sp. after extraction of lipid) with commercial fertilizer at 50%-50% (in terms of nitrogen) as the fertilizer for growing rice crops (IR 36), with solely microalgae biomass waste or chemical fertilizer as baselines for comparison. With the combined fertilizer, the rice crops displayed the best

performance in terms of plant height, tiller number, biomass, and grain yield along with maximum plant dry weight and panicle weight. However, Wichaphian et al. [101] found that mixing the ultrasonication-facilitated aqueous extract from the microalgal (*Chlorella*) biomass residues and commercial fertilizer as the hydroponic fertilizer did not promote lettuce growth in hydroponic systems compared to those grown only with the commercial fertilizer solution, mainly because such combination could result in nutrient imbalance.

Furthermore, the biofertilizer/inorganic fertilizer ratio and microalgal residual biomass compositions perform key roles in influencing plant growth. Maurya et al. [102] compared the effect of composition ratios of microalgal biomass waste (*Chlorella variabilis* or *Lyngbya majuscula*) and commercial urea fertilizer on the growth activity of maize crop (*Zea mays* L.). It was found that the *Lyngbya* biomass residue and urea fertilizer at the nitrogen ratio of 1/3 resulted in the highest yield of maize grains with the highest phosphorus and potassium contents; while *Chlorella* biomass residues and urea fertilizer at the nitrogen ratio of 1/1 led to a higher yield of maize grains, but at the ratio of 3/1 would produce the maize containing more carbohydrate content and experience higher photosynthetic activity in leaves. Similarly, in another study, the combined fertilizers (inorganic fertilizer with *Chlorella* sp., or *Scenedesmus* sp., or *Chlorella* sp.+*Scenedesmus* sp. residual biomass respectively) with different composition ratios were used for growing tomato plant (*Solanum lycopersicum*). It was noticed that when the combined fertilizer consisted of the microalgal residual biomass and inorganic fertilizer at 50%N-50%N ratio, the tomato could achieve 14-95% higher fresh weight and 12-53% higher dry weight compared to those with fertilizers at other combination ratios [103].

On the other hand, as microalgae-derived biomass waste generally contains high organic compounds, an increase of available organic carbon sources in soil was expected after its

incorporation into soil [100, 104]. Organic carbon availability strongly influences the physiochemical properties of soil including nutrients and water retention capacities, soil pH, cation exchange capacities, and soil aggregate stability. In addition, microbial community within the soil utilizes the organic compounds as an energy source, and also takes certain essential organic molecules to accelerate enzymatic activity for their metabolic functions [31, 105]. Such influencing potential was associated with the added microalgal biomass amounts and types as well as soil properties [100, 104].

Some studies have reported that the utilization of microalgae-derived biomass as soil conditioners could improve soil structure and physiochemical properties, leading to an increase of plant production. Lewis et al. [106, 107] reported the incorporation of 1.5-3% of microalgal biomass residues (*Nannochloropsis salina* after lipid extraction) in soil resulted in enhancing soil aggregate formation and organic carbon storage compared to the use of inorganic fertilizer, but leading to increased pH and conductivity. Thus, such soil was more suitable for cultivation of salt tolerant plants, such as foxtail millet (*Setaria italica* L.) and pearl millet (*Pennisetum glaucum* L.). However, during long-term period, the repeated addition of such microalgal residues into soil would cause the increased soil pH and salinity beyond the tolerant levels of plants, reducing plant productivity and encouraging salt leaching from the soil during rainfall to impact surrounding aquatic environments. Combining microalgae biomass residues with other types of organic waste (such as wheat straw) could offer a feasible solution to mitigate the increases of soil pH and salinity.

Nevertheless, the combination of microalgae-derived biofertilizer with inorganic fertilizer would mitigate the agricultural dependence on chemical fertilizers, although the biomass wastes derived from diverse microalgae production processes may lead to dissimilar plant growth patterns due to their various composition profiles. Especially, some of biomass wastes may also contain certain

unfavorable components (such as heavy metals) and extraction chemical residues, which could potentially inhibit the growth-relevant enzyme activity, influence cultivation soil conditions (such as pH, conductivity), and microbial community compositions [96, 102, 103]. Thus, nutrient management practices through the preparation of cocktail fertilizers and optimization of their compositions (proper balance of required elements) are required to maximize the direct use efficiency of microalgae-derived biofertilizers for improving agronomic efficiency and circular economy of microalgal industries.

3.2 Chemical and biological conversion of microalgal wastes

As the microalgal biomass residues contain a great number of organics, they could be converted to carbon-rich biochar via gasification, pyrolysis, and torrefaction. Biochar has a large surface area, abundant micropore structure, rich functional groups, and high stability, thus it has shown a wide range of their applications [108, 109]. Especially, previous studies have addressed that the microalgae residue-derived biochar generally exhibit excellent adsorption capability because it has high nitrogen and mineral contents, as well as special functional groups (such as hydroxyl, carboxylate, amino, phosphate, sulfate) [110-113]. Accordingly, currently reported studies relating to microalgae (*Chlorella vulgaris*, *Spirulina* sp. *Nannochloropsis* sp.) residue-derived biochar products merely focused on their roles as absorbents to mitigate pollutants (such as ionized dyes, tetracycline and enrofloxacin, virus) and as bioenergy source [110-115].

The application of biochar for agricultural sector has been reported, such as soil carbon sequestration and crop production, especially in the agricultural regions requiring mitigation of greenhouse gas emission [116-118]. However, only a few studies relevant to the agricultural application of microalgae residue-derived biochar have been reported. Wang et al. [119] employed

pyrolysis technique to convert microalgal residues (after lipid extraction) to biochar, which contained high amounts of nutrients (potassium, phosphorous, and nitrogen) and thus was suitable for plant cultivation. Yadav et al. [120] also converted microalgal residues (after lipid and carotenoid extraction) to biochar via pyrolysis, which was used as a soil conditioner for cultivation of Mung bean (*Vigna radiata*). The addition of biochar (1-5 g per pot) into soil showed a positive impact on growth and productivity of Mung beans, and a dosage of 4 g per pot was identified as the optimal amount. It shall be recognized that microalgal biomass residues generally have dissimilar compositions, as a result, biochar production processes and the properties of produced biochar (nutrient compositions and amounts, assimilable organic compounds, physical structure) may show a wide range of differences. Thus, when microalgae residue-derived biochar is added into soil, its impacts on soil properties and plant growth as well as optimization of biochar dosage amounts shall be carefully evaluated. As the mechanisms relating to biochar-involved carbon sequestration by soil microorganisms and plant cultivation in agro-environment remains unclear [118], it is necessary to explore the suitable technical pathways for production of microalgae residue-derived biochar towards maximizing their contributions to carbon sequestration and plant production, as well as plant quality considering the superior adsorption behaviors of biochar for reducing the uptakes of heavy metal/micropollutants by plants.

In addition, the microalgal biomass residues can also be utilized to produce valuable products via diverse biodegradation pathways. For example, Khan et al. [121] used the microalgal biomass residues (*Pseudoscillatoria coralii* after extraction of phycocyanin, allophycocyanin, and lipids) to produce α -amylase, which is potentially used as biostimulator to enhance starch degradation during seed germination [122]. Monlau et al. [123] applied the anaerobic digestion process to degrade microalgal (*Chlorella protothecoides*) biomass residues into high value biomolecules such

as amino acids and carbohydrates. Meanwhile, the digestate was collected and used as liquid fertilizer for cultivating wheat and tomato plants, which displayed similar germination rate and dry biomass amount compared to the commercial fertilizer after 21~28-day soil-based growth. Nevertheless, the energy efficiency for conversion of biomass residues to agricultural-used products, quantity and quality of plants cultivated with these products shall be carefully evaluated before selection of proper downstream processes.

Although pyrolysis-based conversion and biological processes for nutrient recovery from microalgal biomass residues have paramount significance considering economic feasibility and sustainability [124, 125], they have their own advantages and challenges. Major advantages of pyrolysis-produced biochar include that (1) biochar shows highly structural stability in soil, which allows it to perform long-term carbon sequestration, enhancing soil quality for improving plant growth; (2) biochar contains high amounts of nitrogen, phosphorus, potassium, making it ideal fertilizers to promote plant growth [126]. However, pyrolysis requires high energy consumption and may induce volatilization of nitrogen beyond certain pyrolysis temperature [127]. Whereas, biological pathways such as anaerobic digestion of microalgal biomass would preserve nitrogen in the form of ammonium, which could be efficiently delivered to the soil and directly utilized by plants to conduct amino acid metabolism compared to other types of nitrogen forms. Furthermore, biological processes are generally more accessible and cost-effective than pyrolysis-based conversion [128]. However, biological-processed biofertilizers as commercial products experience a shorter shelf life, thus requiring more precise storage conditions.

4. Circular economy and future perspectives

Microalgae-derived biorefinery industries for manufacturing high-value compounds (e.g., astaxanthin, carotenoids) are expanding globally. Towards achieving the United Nations Sustainable Development Goals (such as Goal 6: clean water and sanitation for all; Goal 8: decent work and economic growth; Goal 12: responsible consumption and production; Goal 13: climate action), the “circular loop” concept has been adopted for microalgae biorefinery industries, including efficient utilizing water/energy, minimizing waste generation, and maximizing resource recovery [129, 130]. Many advanced technologies for improvement of productivity and resource recovery are being implemented in microalgae industries, and the key factors relating to process operation, economic growth, and environmental impacts are being identified. Especially, several pilot-scale studies have demonstrated the technical feasibility of recycling spent cultivation medium for microalgal growth [39, 131]. In addition, utilization of wastewater and/or flue gas-derived CO₂ as nutrients for cultivation of microalgae has been recognized as circular economy models for microalgae biorefinery industries [132-135]. Thus, reuse of recycled water from microalgae harvesting process and/or conversion of solid waste to other valuable co-products could be integrated with above-mentioned models in order to simultaneously achieve circular economy and zero-waste goals in microalgal biorefinery industries (Figure 5) [136].

As presented in this review, the techniques for treatment of aqueous/solid waste derived from microalgal industries towards agricultural practices have been well documented, however, current technical development is still at lab-scale and faces several key challenges. For example, (1) the treated microalgae processing wastewater may contain insufficient amounts or improper ratios of nutrient elements (N, P, K) for plant cultivation in most scenarios, which requires additional supplementation of commercial fertilizers; (2) During chemical-based microalgal harvesting and/or extraction of valuable products from microalgae biomass, certain chemical compounds may

be present in the biomass residues, which could negatively influence both plant productivity and quality by interfering with metabolic enzyme synthesis, reducing nutrient uptake and photosynthetic activity, causing oxidative stress, etc. [137]; (3) The microalgal biomass residues still contain complex carbohydrates such as cellulose and lignin that could delay the release of available nutrients for plant uptakes due to their slow hydrolysis behaviors; (4) The use of microalgal-based waste streams/residues may cause increases of pH and conductivity in soil/hydroponic systems, resulting in osmotic stress on plant roots and negative impacts on the rhizosphere microbial community that could limit plant nutrient absorption and uptake activity.

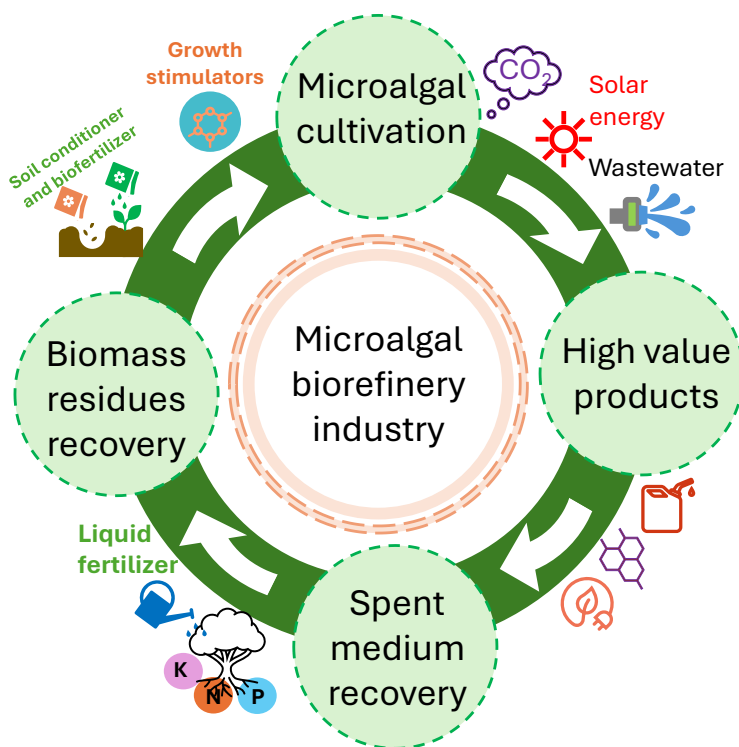


Figure 5. Circular economy in microalgal biorefinery industries

In this regard, future efforts shall be put on (1) the developments of pre-treatment steps (such as membrane filtration) or detoxification processes (such as biological/chemical conversion processes) to remove unfavorable components from the microalgal processing

wastewater/biomass residuals, maximizing the resource utilization efficiency during their applications in agriculture practices; (2) integration of microalgae biorefinery with “waste-to-food” concept at large-scale systematic level, i.e., combining multiple resource recovery pathways (nutrients, water, organics, etc.) from diverse types of microalgae-derived waste (such as used cultivation medium, extraction solution, solid biomass residues, etc.) for their reuses in agricultural sector. Thus, the techno-economic-environmental feasibility analysis of resource recovery from microalgal industries for agricultural sector and optimization of operating conditions under a complex multi-stage process are crucially important [136, 138]. Currently developed data-driven techniques, such as artificial intelligence (AI) techniques, would offer efficient and reliable multi-objective optimization algorithms to (1) identify suitable treatment processes and their key operation parameters towards maximizing resource recovery from microalgae-derived wastewater and biomass residues, and (2) predict cultivation conditions, favorable plant types, and plant yields. This could allow microalgal biorefinery industries to readily adopt advanced techniques and fill practical gaps in achieving circular economy [139, 140].

5. Conclusion

The microalgal processing wastewater and biomass residues contain valuable resources, such as water, macronutrients (N, P, K), organics, micronutrients, and bioactive compounds. Both (1) recovery of liquid fertilizers from microalgal processing wastewater by membrane processes or from microalgal biomass residues by aqueous extraction/biodegradation, and (2) direct use/conversion of microalgal biomass residues as solid biofertilizers provide promising approaches to reduce dependency on expensive synthetic fertilizers in agriculture. Especially, certain components in microalgal processing wastewater and biomass residues displayed their capabilities as soil conditioners and biostimulators, which could improve both productivity and

quality of plants in both soil- and hydroponic-based cultivation. This review underscores the significant potential of microalgal biomass residues (after extraction of valuable products instead of fresh microalgal biomass) and wastewater as agricultural bio-inputs, particularly in the framework of sustainable practices integrated with circular economy principles.

However, several challenges remain during their applications for agricultural practices, e.g., unfavorable pH/conductivity-induced osmotic stress on plant roots, the presence of heavy metals or residual chemicals. Additionally, conversion of biomass residues to valuable products for agricultural practices shows its technical feasibility, but economic analysis and environmental impact assessment at a system level are still limited. Thus, future focuses shall be emphasized on (1) examining long-term effects of recovered liquid and solid biofertilizers on soil health, nutrient cycling, microbial community shifts, and accumulation of unfordable contents (such as heavy metals, growth inhibitors) in soil and plants, and identifying suitable solutions to conquer negative impacts; (2) designing proper pathways to integrate resource recovery processes with microalgal biorefinery processes, and performing systematic life cycle assessments and techno-economic analyses in order to guide process integration and achieve maximum economic benefits with minimum environmental impacts.

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