Harvested Microalgal Biomass from Different Water Treatment Facilities—Its Characteristics and Potential Use as Renewable Sources of Plant Biostimulation

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Received: 23 October 2020; Accepted: 26 November 2020; Published: 27 November 2020

Abstract: Surface characteristics, physicochemical properties, functional groups, and bioactive compounds of microalgal biomass (MB) samples harvested from various wastewater treatment facilities (WTFs) were investigated to evaluate the reuse feasibility of MB as a potential renewable source of plant biostimulation. Mixtures of the microalgae cells and fine particles (i.e., silt, clay, suspended solids, extracellular organic matter, humus substances, natural organic matter, etc.) were complexed inside MB samples. MB samples harvested and air-dried under natural conditions investigated in this study can have relatively well-preserved cellular morphology as well as chemical substances such as carbohydrates, proteins, and fatty acids based on SEM-EDS analysis. A broad form of the amorphous cellulose rather than a distinct crystalline was observed from FTIR analysis, indicating that the middle spectrum of glucose and starch hydrolysate exist in MB samples. A wide array of chemicals (i.e., Undecane; Heptadecane; Hexadecanoic acid, methyl ester; and Methyl stearate, phenolics, and fatty acids) extracted from MB samples were involved in signaling plant response to abiotic stress, plant growth and biomass with MB samples were greater than those without MB samples. Thus, mixtures of nutrients, minerals and algal biomass in wet and dried MB samples can be beneficially reused as biostimulants in agricultural area after simple processes such as composting, microbial fermentation, and extraction. Further study is warranted to elucidate the effect of useful ingredients in MB harvested from on-site coagulation/flocculation processes on the soil environment as bio-fertilizers.

Keywords: agriculture; surface characteristics; physicochemical properties; functional groups; bioactive compounds; microalgal biomass (MB), biostimulation

1. Introduction

Algae (i.e., microalgae and macroalgae) are the photoautotrophs that can achieve high photosynthetic efficiency by fixing CO₂ in water or by absorbing nutrients for cell growth, and can produce a wide range of green compounds as a ‘living biorefinery’ [1–3]. In general, green compounds from algae can be classified into primary metabolites such as carbohydrates materials (e.g., starch, fermentable sugars, etc.) and lignin, and secondary metabolites (i.e., high-value green chemicals) such as fatty acids, phenolic substances, steroids, tannins, esters, alkaloids, and furan molecules [1,4,5]. Therefore, algae have been recently recognized as one of the promising resources...
in various fields such as biofuels, medicine, and the global food system [6]. Since microalgae can grow in independent cell units by small particles with many advantages (i.e., fast doubling time, high productivity, and favorable absorption of nutrients in water) and are relatively easy to further process [7]. MB is available for use as a resource with many advantages in various materials and industries.

The organic residue of MB is available for use as an agricultural resource, as well as the major parts of biorefinery [8]. For example, many researchers have reported that algae can be a good potential biofertilizer [8–10], although nitrogen and phosphorus derived from traditional biorefinery can be manufactured from the anaerobic digestion of other biomass and wastes. Therefore, if more efficient use of MB is developed, previous mineral fertilizers can be replaced with these ecological biofertilizers using MB for the renewable and sustainable agricultural industry. Despite the biological high-value of MB, research in the agriculture field using MB is still lacking. However, the agricultural use of high-value products from the primary and secondary metabolites of MB can be one of the promising areas of the industry, considering that MB as an agricultural resource presents both possibility and opportunity as sources of biofertilizer.

Recently, there have been growing attempts to recycle MB in various fields. For example, several studies have reported that wastewater treatment with high rate algal ponds not only removes nutrients, but also enables the economical production of biofuels [7,11–13]. Furthermore, some studies have suggested the use of dried MB or its extracts as fish feed or dietary additives [14,15]. Also, algal-related substances have contributed to both growth and feed use of cultured fish via the effective assimilation of protein, physiological activity, stress reaction, tolerance to starvation, increase in fatty acids, disease resistance, and improvement of meat quality [16,17]. Finally, some researchers have also attempted to reuse harmful algal biomass as high-performance electrodes for sodium-ion batteries [18].

However, algal blooms in the stagnant water zones with high nutrient levels can cause problems in water resources by releasing toxins causing illnesses for animals and humans [19]. In particular, harmful algal blooms (HABs) by some specific species foaming scum or mats on the water surface and causing adverse effects on human life, aquatic ecosystem and aesthetic environment are becoming more frequent with climate change [20–23]. Also, these HABs have caused various problems with surface water quality including color, taste/odor, sediment, and toxic substances such as microcystin and anatoxin. [24,25]. Since the HABs induced by eutrophication are important global issues regarding water quality and potential use as a feedstock for biofuel, both proactive and responsive operation of water treatment systems for effective control [20] and useful bioremediation technique [26–28] from algal biomass are required for sustainable water quality management including microalgae harvesting and reuse. Thus, in the future, developing recycling measures of MB as a valuable resource is required for sustainable water quality management and renewable reuse of harvested HABs.

In this study, we recognized the value of MB derived from HABs in freshwater ecosystems as a potential renewable source of plant biostimulation and conducted preliminary testing for agricultural applications. Previous studies have focused on identifying the characteristics of allogenic organic matter (AOM) pertaining to the water treatment processes of coagulation, sedimentation, filtration, and disinfection, and have mostly conducted at the laboratory- or pilot-scale [29,30]. In this study, we collected MB samples from various field-scale surface water treatment facilities (WTFs) by harvesting HABs on-site during the period of freshwater algal bloom. We also focused on analyzing both surface characteristics and physicochemical properties of MB samples to evaluate the feasibility of reuse of MB as a potential renewable source of plant biostimulation. Thus, the specific objectives of this paper are to (1) investigate the surface characteristics of MB samples from various WTFs, (2) compare the mineral composition and structures of MB samples from various WTFs, (3) investigate functional groups and bioactive compounds of MB samples from various WTFs, and (4) evaluate the potential application of dried MB as biostimulant. We finally discuss the potential beneficial reuse of the MB derived from HABs to agricultural fields based on these properties analyzed in this study. Then, future research relevant to microalgae-based biostimulants is proposed, encompassing the effective bioactive compounds as well as their applications for sustainability in agriculture.
2. Materials and Methods

2.1. Algae Harvesting Methods

In this study, MB samples were obtained from the on-site algae collecting systems placed in the eutrophic freshwater of three WTFs. The three types of WTFs includes algae harvest ship (AHS), dissolved air flotation (DAF) of the pilot plant, and on-site microbubble ship (OMS), respectively, as displayed in Table 1 and Figure 1. The GPS coordinates of each in situ point are 36°0′35″ N 128°56′59″ E in AHS, 36°0′42″ N 128°57′07″ E in DAF, 37°30′40″ N 127°06′19″ E. The coagulation-flotation processes were applied in all three WTFs, and the harvested MB samples were classified as MB₁ for AHS, MB₂ for DAF, and MB₃ for OMS, respectively. Both MB₁ and MB₂ were obtained from a dam (open water and edge), and MB₃ was obtained from an artificial lake where eutrophication causes severe HABs (see Figure 1). Initial analysis of the water content of MB samples resulted in 90% in both MB₁ and MB₂, and 95% in MB₃. The sampling period was August to September in 2018.

Table 1. Summary of harvested MB samples in the different surface water by various WTFs.

| WTF                        | Coagulant                | Capacity (ton day⁻¹) | Description                                                                 | Harvested Sample | Site (Date)       |
|---------------------------|--------------------------|----------------------|-----------------------------------------------------------------------------|------------------|------------------|
| Algae harvest ship (AHS)  | Plant mineral composite  | 30,000               | Recovery of surfaced algal sludge from the water after the coagulant is applied to the algal bloom area | MB₁             | OD in site B     |
|                           | composite (PME)          |                      |                                                                             |                  | (August 2018)    |
| Dissolved air flotation (DAF) | Poly aluminum           | 240                  | Water by algal bloom at the waterbody edge is transferred to the pilot plant to separate the algal solid from the water using the cyclonic-DAF method | MB₂             | ED in site B     |
|                           | (PAC)                    |                      |                                                                             |                  | (August 2018)    |
| On-site microbubble ship (OMS) | Al₂(SO₄)₃       | 30,000               | Algae sludge is collected on the water surface via the coagulation-floating process using microbubbles in a compact facility on-site | MB₃             | AL in site C     |
|                           | (Alum)                   |                      |                                                                             |                  | (September 2018) |

Each WTF has distinct scope and responsive operation of water treatment systems for effective control of algal blooms. For example, AHS is used for rapid response to algal blooms in open water, enabling direct removal of microalgae in large streams, reservoirs, and lakes. DAF is used in pilot plants to remove algae concentrated at the edge of the dam. In this study, conventional DAF mechanism (i.e., the simultaneous reaction of coagulants and air microbubbles to float and separate the coagulated mixtures) and floating vehicle-type device for easy harvesting and transportation were applied. OMS is equipped with compact on-board devices including a silt protector and conveyor belt and is easy to use in small- and medium-sized artificial lakes in the urban areas. For coagulants, plant mineral composites (PMCs) manufactured by A company was used for AHS since PMCs manufactured by extracting minerals and plant materials were reported to be effective for algae coagulation in previous studies [31]. Whereas commercialized PAC (poly aluminum chloride, 10% Al₂O₃) was used for DAF, commercialized Al₂(SO₄)₃ (alum, 8% Al₂O₃) was used for OMS.
2.2. Analysis of Water Quality

Three points for water quality measurement were designated: the open water of the dam (OD) and the edge of the dam (ED) in site B, and an artificial lake (AL) in site C. A real-time multiple water quality monitoring sensor (ProDSS, YSI, Yellow Springs, OH, USA) was used to measure water temperature, pH, dissolved oxygen (DO), DO saturation, electrical conductivity (EC) and turbidity. Samples for water quality analysis were collected using a Van Dorn sampler. A 4-L polyethylene bottle was used to collect each water sample and was transported to the laboratory for analysis. The analyzed physicochemical water quality parameters were suspended solids (SS), total organic carbon (TOC), biochemical oxygen demand (BOD), chemical oxygen demand (COD$_{m}$), total nitrogen (TN), ammonia (NH$_{3}$$+$-N), nitrate (NO$_{3}$$-$-N), total phosphorus (TP), phosphate (PO$_{4}$$^{3-}$-P), and chlorophyll-$a$ (Chl-$a$) and analysis was performed based on the standard methods [32]. Details regarding both sites and harvested MB samples are provided in Table 1.

2.3. Analysis of the MB Samples

Microalgae samples in surface water were collected using a plankton net (mesh size = 60 $\mu$m) and harvested fresh MB samples on-site at each WTF are displayed in Figure 1. Collected microalgae samples were fixed in glutaraldehyde (1–2%) for immediate laboratory analysis. The species of microalgae were identified using an optical microscope (Nikon, Ni-U Co., Tokyo, Japan) in a Sedgewick-Rafter counting chamber. Cell counts were performed at various magnifications (e.g., within 1000×) to classify Family level for taxonomic composition. The surface morphology and element compositions of all naturally dried MB samples for seven days were investigated using scanning electron microscopy (SEM) from JEOL-FE-SEM (JSM-6701F, JEOL Ltd., Tokyo, Japan) and Oxford (EDS7585, Oxford Ins., UK). To determine the characteristics of structure and crystal lattice, X-ray diffraction (XRD) analysis was obtained from Rigaku (Ultima IV, Rigaku Co., Tokyo, Japan) using Cu Kα irradiation (40 kV and 100 mA) scanning from 10 to 90° at a scan speed of 4° min$^{-1}$. Fourier transform infrared (FTIR) analysis was performed using Bruker (Vertex 70, Bruker Co., Billerica, MA, USA) to investigate functional groups of MB samples. The dried MB samples were mixed with KBr pellets and compressed to form a disk. Before the direct qualitative analysis of MB samples using gas chromatography (GC), sonication extraction on dried MB samples in ethanol for two hours was performed. Then, the analysis of organic compounds was performed using a gas chromatography/mass spectrometry (GC/MS) instrument from Agilent Technologies (7890B-5977A, Agilent Tech., Santa Clara, CA, USA). Organic compounds were separated on a 30-m-long × 250 $\mu$m,
inner-diameter, 1.4-μm DB-624 column, high-purity (99.999%) helium flowed through the column at 1 mL min⁻¹, and the temperatures for the inlet and the detector were 250 and 280 °C, respectively. The oven temperature was maintained at an initial value of 40 °C for 5 min, raised at 10 °C min⁻¹ to 250 °C. The qualitative analysis was performed based on the NIST17.0 mass spectrometry database, linear retention index and mass spectrogram of target compounds, and the quantitative analysis was performed using the internal calibration standards. Finally, we focused on the fatty acids, which are representative secondary metabolites of MB, as the important green compounds.

2.4. Application of the MB Samples on Plant Growth

We evaluate the direct effect of the harvested MB samples on plant growth. To compare the impact of MB samples as a biostimulant, five experimental groups including control (commercial topsoil), composted sawdust, composted MB sample (11.7% w/w), composted MB sample (21.6% w/w), composted MB sample (37.6% w/w) were prepared. For the plant growth experiment, the experimental groups except for the control were cultivated in a greenhouse at 25 °C as a mesocosm under 250 kg 1000 m⁻² conditions. Perilla sp. was used as an experimental plant for growth development for 14 days. Fresh weight and leaf conditions were monitored and evaluated for productivity and harmful effects of plan growth. The collected data were statistically evaluated using the R program software (version 3.6.3).

3. Results and Discussion

3.1. Water Quality and Algal Composition

The results of the water quality analyses were summarized in Table 2. Since both OD and ED are located in the same rural catchments and differ only in sampling points inside the dam (i.e., open water and dam edge, respectively), the overall water quality was similar. However, the levels of Chl-a were significantly different with values of 91.7 μg L⁻¹ (OD) and 46.4 μg L⁻¹ (ED), respectively. Considering that OD corresponds to the deepest site (approximately 5m depth) in the middle of the reservoir, where green tide of concentrated algae was observed at the water surface due to the accumulation of algae by both wind and water currents, the levels of Chl-a were greater. Another difference found in water quality was NH₃+N level higher in the ED than that in the OD, and this difference might be attributed to the direct input of soil particles or fertilizers into the ED from the watershed.

The lower Chl-a concentration in AL relative to that in OD or EM suggests a relatively small microalgae biomass, which was also evidenced by lower levels of pH and DO as byproducts of photosynthesis. According to the Korean “Algae Alert System (AAS)” by the Korea government issued in 2015 [33], these values of Chl-a ranged between “advisory” and “warning” levels. Thus, on-site WTFs were operated proactively. Across the three sampling sites, the characteristics of water quality were generally consistent with those during the summer season in Korea [34] with relatively high levels of organic compounds and nitrogen in OD and ED and phosphorus in AL (see Table 2).

The compositions of the microalgae species in the three MB samples were different in MB₁: (Cyanobacteria 97.8%, Diatom 1.8%, Green algae 0.4%), MB₂: (Cyanobacteria 89.3%, Diatom 10.4%, Green algae 0.3%), and MB₃: (Cyanobacteria 76.9%, Diatom 3.1%, Green algae 19.8%, others 0.2%), respectively. During the sampling period, HABs by cyanobacteria cells corresponding to the “warning” level of the AAS occurred at all sampling sites, and cyanobacteria were dominant at all sampling sites during the summer [33,34]. The microscopic analysis also proved that the Microcystis sp. of cyanobacteria was dominant in all MB samples harvested in this study.
Table 2. Measures of water quality from the three water samples collected at different sampling sites.

| Description          | OD   | ED   | AL   |
|----------------------|------|------|------|
| Temperature (°C)     | 27.6 | 27.7 | 26.0 |
| pH                   | 9.6  | 9.5  | 8.9  |
| DO (mg L\(^{-1}\))  | 12.0 | 11.6 | 9.2  |
| DO saturation (%)    | 149  | 148  | 115  |
| EC (μS cm\(^{-1}\)) | 114  | 124  | 152  |
| Turbidity (NTU)      | 17.8 | 17.3 | 6.7  |
| SS (mg L\(^{-1}\))  | 6.4  | 6.6  | 3.5  |
| TOC (mg L\(^{-1}\)) | 5.8  | 5.3  | 2.6  |
| BOD (mg L\(^{-1}\)) | 4.0  | 3.9  | 1.5  |
| COD\(_{M_0}\) (mg L\(^{-1}\)) | 12.0 | 9.6  | 3.2  |
| TN (mg L\(^{-1}\))  | 0.97 | 1.16 | 0.79 |
| NH\(_4\)-N (μg L\(^{-1}\)) | 0.07 | 0.46 | 0.14 |
| NO\(_3\)-N (μg L\(^{-1}\)) | ND \(^a\) | ND \(^a\) | 0.43 |
| TP (μg L\(^{-1}\))  | 0.047 | 0.052 | 0.062 |
| PO\(_4\)^{3-}-P (μg L\(^{-1}\)) | ND \(^a\) | ND \(^a\) | 0.035 |
| Chl-a (μg L\(^{-1}\)) | 91.7 | 46.4 | 19.5 |

\(^a\) ND: no data; OD: open water of the dam; ED: edge of the dam; AL: artificial lake.

3.2. Morphology and Structure of Dried MB

The SEM images displaying original structure of the algae in the dried MB samples are shown in Figure 2. A solid form with unclear boundaries in all MB samples was observed. Although the dominant species of the MB samples were cyanobacteria (e.g., *Microcystis* sp., *Anabaena* sp., etc.) in microscopic view, cell walls in MB samples were not clearly found in the SEM image. Instead, the shells of diatoms were observed in every MB sample and were relatively clear in the MB\(_2\) (see Figure 2c,d). While typical diatom shells are strong and made of silica preserving both shape and structure of cells, both shape and structure of cyanobacteria are not relatively distinct after harvesting and drying processes of MB samples. Since coagulation-flocculation processes in water occurred through the interaction between positively charged salts and negatively charged matters, mixtures of the microalgae cells and fine particles (i.e., silt, clay, suspended solids, extracellular organic matter, humus substances, natural organic matter, etc.) were combined at the same time. As a result, the surface of flocs was found to be relatively rough and dense after drying.
Previous studies have reported that overall morphology was maintained after carbonization of cyanobacteria \textit{(Nostoc sp.)} at varying temperatures (700 to 1000 °C) and only size reduction as well as wrinkles in the spherical cell surface was observed in some microalgae [18]. Moreover, it has been reported that the carbonized microalgae cells were still continuously connected by a carbon filament layer and gelatinous filaments, suggesting that the basic cell morphology as well as chemical substances were preserved to some extent even after such thermal carbonization processes [18]. In this sense, microalgae products harvested and simply air-dried under natural conditions without undergoing harsh processing applied in this study can have relatively well-preserved cellular morphology as well as chemical substances such as carbohydrates, proteins, and fatty acids. Thus, MB samples containing these bioactive compounds with high nutritional values can be used as effective renewable sources for plant biostimulation in agriculture.

EDS analysis was performed to determine the elemental composition of aggregated algal flocs. The EDS results displayed that the surface composition of major elements such as C, O, Al, and Si was evenly distributed (see Table 3 and Figure 3). The main components of carbon-based organisms were C and O and well distributed throughout the surface of the MB samples (weight 67.58–84.49%). Among harvested MB samples, Si composition is relatively high in MB2 and MB3, which is mainly attributed to greater contents of diatoms in MB samples. The range of Al composition across the MB samples was 7.95–11.17% mostly resulting from the aluminum-based coagulants. Whereas relatively low weight fraction and uneven distribution of Al were found in MB3, greater weight fraction and even distribution of Al were found in MB1. Considering that the dominant algal specie in MB1 was cyanobacteria, the adhesive sorption of aluminum hydrolysis to flocculated cyanobacteria could be superior due to the presence of gas vacuoles and greater amounts of extracellular polymeric substances of cyanobacteria. Consistent with these results, analysis of the \textit{Microcystis} sp. cell surface after coagulation revealed relatively high levels of Al (14.27%) in the algal flocs, indicating that

**Figure 2.** SEM images of harvested and dried MB samples collected directly from the three water treatment facilities. (a,b) AHS; (c,d) DAF; (e,f) OMS.
aluminum hydrolysis can be easily coagulated with the extracellular polymeric substances of cyanobacteria [35–37]. In addition, trace amounts of Fe, Ca, S, K, Mg and Mn were observed in all MB samples.

Table 3. EDS results of the harvested and dried MB samples in the surface water of the three water treatment facilities.

| Element | MB1  |   | MB2  |   | MB3  |   |
|---------|------|---|------|---|------|---|
|         | Weight (%) | Atomic Level (%) | Weight (%) | Atomic Level (%) | Weight (%) | Atomic Level (%) |
| C       | 38.78 | 48.57 | 23.44 | 33.52 | 41.32 | 51.50 |
| O       | 46.23 | 43.47 | 44.14 | 47.39 | 43.17 | 40.40 |
| Al      | 11.17 | 6.23  | 9.52  | 6.06  | 7.95  | 4.41  |
| Si      | 1.89  | 1.01  | 18.35 | 11.22 | 5.48  | 2.92  |
| Fe      | 0.59  | 0.16  | 2.06  | 0.63  | 0.78  | 0.21  |
| Ca      | 0.29  | 0.11  | 0.88  | 0.38  | 0.52  | 0.19  |
| S       | 0.56  | 0.26  | 0.60  | 0.32  | 0.40  | 0.19  |
| K       | 0.18  | 0.07  | 0.86  | 0.38  | 0.20  | 0.08  |
| Mg      | 0.07  | 0.05  | 0.15  | 0.11  | 0.18  | 0.11  |
| Mn      | 0.25  | 0.07  | ND    | ND    | ND    | ND    |
| Total   | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

* ND: no data.

Figure 3. EDS mapping analysis results of the MB samples. (a) SEM image (×2000); (b) distribution of the various elemental composition on the algal surface; (c) C; (d) O; (e) Al; (f) Si.

Although the coagulants applied in this study had different dosage and ingredients applied in different period of HABs, no significant difference in the type or elemental composition of the harvested MB samples was found. Since the amount of MB samples was greater than the amount of input coagulants in the dried MB samples, dried MB samples may contain rich nutrients (macro and micro) and minerals along with the dense algal flocs. Even though both surface and elemental composition of MB samples may have been affected by the physicochemical properties of the on-site water and both type or efficiency of WTFs, mixtures of nutrients, minerals and algal biomass in harvested and dried MB samples can be beneficially reused in the agricultural area after simple
processes such as composting, fermentation and extraction due to the presence of rich nutrients (macro and micro) and minerals as biostimulants.

3.3. Mineral Composition and Structure of Dried MB

As displayed in Figure 4, a single broad amorphous peak was observed from XRD results and this peak is a complex form of closely overlapped peaks observed in natural organic matters [38]. The following characteristics were found across all MB samples. First, the MB samples displayed the characteristics of SiO$_2$. The XRD results from MB$_1$ and MB$_3$ displayed a SiO$_2$ peak ($2\theta = 22.5^\circ$) due to the presence of the amorphous structure of synthesized silica. Consistent with this study, similar SiO$_2$ peaks or broad amorphous characteristics are also observed in much organic sludge including microalgae [39]. The greater intensities of SiO$_2$ peaks in MB$_2$ and MB$_3$ than those in MB$_1$ suggest the greater ratio of diatoms with strong silica shells. Consistent with the results of EDS mapping analysis demonstrating the outer layer of diatoms mainly composed of Si (see Figure 3f), an increase in Si content with increasing diatoms biomass is expected, consistent with a previous study [40].

![Figure 4. XRD patterns of harvested MB samples (a) MB$_1$; (b) MB$_2$; and (c) MB$_3$. ■ denotes a SiO$_2$ peak, ○ denotes an Al peak, and ◆ denotes a cellulose I peak.](image)

As is also presented in Figure 4, the characteristics of cellulose I peak known to exhibit broad peaks (10–30°) including 14.7°, 16.8°, 20.5°, 22.7°, and 34.4° [41] were observed in all MB samples. These XRD results showed a broad form similar to the amorphous cellulose rather than a distinct crystalline. Since similar broad peaks from cellulosics derived from plants were also observed [42], these XRD results indicate that various natural organic matters originated from plant and microalgae are assorted in MB samples. In addition, these cellulosics can preserve nutrients inside the cell walls until being decayed [43] and can be readily processed into fertilizers via composting processes [44,45].

Preservation of the cellular morphology of microalgae by cellulose I is very critical since early loss and leakage of beneficial compounds can occur when the cell is destroyed. As shown in Figure 1b from the microscopic analysis results, most cells were intact right after coagulation-flocculation processes, suggesting that the on-site coagulation-flocculation processes did not adversely affect the cellular structure. Thus, the intracellular substances can be preserved to a certain extent even after MB harvesting. Moreover, both degrees of crystallization and cellulose ratio are lower in microalgae than in lignocellulosic biomass, which allows easier physicochemical destruction of the cell wall and serve as an advantage in later additional various processes (physical, chemical, and biological) for saccharification or the extraction of biologically active compounds [39]. Thus, MB samples harvested
in the present study may retain higher levels of bioactive compounds and thus serve as a potential biostimulant in agriculture.

3.4. Functional Groups of Dried MB

FTIR analysis can be used to distinguish the unique functional groups of microalgae with various functional groups depending on the species composition. For example, the FTIR of *Microcystis aeruginosa* (cyanobacteria in freshwater) based on growth conditions shows a high correlation with the micromolecular pools at 1645 cm\(^{-1}\) (amid I band) and 1255 and 1223 cm\(^{-1}\) (phosphate ester band), while the *Protoceratium reticulatum* (Dinoflagellates in seawater) shows high correlation at 1707 cm\(^{-1}\) (amid I band), 1234 cm\(^{-1}\) (phosphate ester band), and 1034 cm\(^{-1}\) (carbohydrate band). Also, the contribution of functional groups could be affected by the chemical modification of the groups depends on growth conditions [46]. Overall, the microalgal functional groups harbor species specificity, which is closely linked to physiological and ecological characteristics as well as survival strategies to the environment [47].

Figure 5 displays the results of FTIR spectral analysis from MB\(_1\) to MB\(_3\). The comparison of FTIR spectra of MB samples displayed a similar pattern of absorption bands commonly observed in aquatic organic substances with overall broad band characteristics [48,49]. The broad IR band at 3400 cm\(^{-1}\) corresponds to O-H stretching, which occurs due to the atomic force of H, O, and -NH. O-H stretching was observed in a very broad distribution [50] and the 3400–3200 cm\(^{-1}\) region, in particular, is known to be composed of water impurities and oximes [51,52]. The IR band at 2950–2850 cm\(^{-1}\) represents the aliphatic C-H stretching group and the bands at 2930 and 2850 cm\(^{-1}\) represent the methyl and methylene groups based on CH\(_3\) and CH\(_2\), respectively [51].

![FTIR spectra of MB samples](image)

**Figure 5.** FTIR results of the harvested MB samples. (a) MB\(_1\); (b) MB\(_2\); and (c) MB\(_3\).

The IR band of 1700–1400 cm\(^{-1}\) is usually referred to as the peptide amide groups as a major reference point for estimating protein content [53,54]. Specifically, it is divided into the amide I band (around 1660–1653 cm\(^{-1}\)) and the amide II band (around 1575–1400 cm\(^{-1}\); [55,56]), both of which were observed in MB samples. In addition, the phosphodiester backbone of DNA, RNA (1398–1230 cm\(^{-1}\)), and N-H of amides associated with protein (1650–1540 cm\(^{-1}\)) may also be included in the broad IR band in part, but no distinct peaks have been identified due to overlap [57,58].

The IR band of 1200–900 cm\(^{-1}\) is the representative carbohydrate region [54], which generally represents C-O stretching. Although band assignment varies with position and intensity [59], there was a prominent peak at around 1088–1038 cm\(^{-1}\), indicating that the middle spectrum of glucose and starch hydrolysate exist in MB samples [51]. Previous studies have reported that this IR region corresponds to compounds such as siloxane and polysaccharides [51,57] or ether compounds [52]. Another study demonstrated a similar peak point (1050 cm\(^{-1}\)) and a strong correlation with carbohydrate content and biomass [60]. In the case of the *Chaetoceros muellerii*, a floating type of
diatoms, a strong peak at 1078 cm⁻¹ and SiO stretching vibration of the silica frustules around the cells were observed [61,62]. Given these findings from IR spectra, the relevant bands are recognized as direct biomass of diatoms.

Although the direct influence of individual algal functional groups is not fully verified in this study, algal biomass as additives with the high nutrient value could yield positive results [i.e., an increase in organic compounds in soil, soil water holding capacity, plant weight, seed germination, nitrogen fixation, ameliorating nitrogen demand, and pigments (Chl-a and b, carotenoids, etc.)] as bio-stimulants [9,41,55,63–65]. Furthermore, the algal carbohydrate region in MB samples is mostly polysaccharides and therefore easy to saccharification, which contributes to the growth and dominance of microorganisms enriching the soil during composting [43]. These microorganisms use polysaccharide-derived substrates such as glucose for succession and contribute to soil fertility through the further decomposition of polymer fibers such as cellulose, hemicellulose, and pectin [43,66]. Therefore, MB-derived microbial fertilizers evaluated in this study are expected to have both eco-friendly and sustainable impacts on soil and plants to enhance plant performance, resilience to environmental stress, and nutrient use efficiency [56].

3.5. Bioactive Compounds of Dried MB

To identify the chemical ingredients in MB₂ and MB₃, GC/MS was applied. From GC/MS results, both MB samples contained complex mixtures of organic compounds with different molecular structures and molecular weights. Whereas 17 chemicals were identified in MB₂, 23 chemicals were identified in MB₃. A total of four hydrocarbon-based fatty acids compounds (Undecane; Heptadecane; Hexadecanoic acid, methyl ester; and Methyl stearate) were observed to be dominant (see Table 4 and Figure 6). Consistent with these results, these compounds have also been identified as green chemicals in many previous studies using microalgae (Oscillatoria sp., Nannochloropsis sp., Chlamydomonas reinhardtii, and Chlorella vulgaris) [52,67–69] and macroalgae (Laurencia caspica) [70]. In the present study, the largest peak area was identified as Undecane (31.84%) in MB₂ and Hexadecanoic acid and methyl ester (22.56%) in MB₃, respectively.

Table 4. Major chemicals of the harvested MB² and MB³ samples identified from GC/MS analysis.

| Compound                       | Formula         | Peak Number | R.T. (min) | MW (g mol⁻¹) | Class/Subclass          |
|--------------------------------|-----------------|-------------|------------|--------------|-------------------------|
| Undecane                       | C₁₁H₂₂          | (7), (7)    | 7.444      | 156          | Saturated hydrocarbons/Alkanes |
| Heptadecane                    | C₁₇H₃₆          | (15), (17)  | 12.823     | 240          | Saturated hydrocarbons/Alkanes |
| Hexadecanoic acid, methyl ester| C₁₇H₃₆O₂        | (16), (21)  | 14.420     | 270          | Fatty Acyls/Fatty acid esters |
| Methyl stearate                | C₁₇H₃₆O₂        | (17), (23)  | 15.689     | 298          | Fatty Acyls/Fatty acid esters |

*a R.T.: retention time; *b MW: molecular weight.

Figure 6. GC/MS result of the harvested MB samples (a) MB² and (b) MB³. R.T. means retention time.

Although the individual functions of the aliphatic compounds in MB samples have not been clearly defined, many studies have insisted on the usefulness of the abundant secondary metabolites
of extracted algal-based phytochemicals. Ragunathan et al. [71] reported that \(n\)-Hexadecanoic acid showed antioxidant, nematicide, and pesticide effects; Undecane, 2-methyl- showed antimicrobial function; and Hexadecanal showed antibacterial and antioxidant effects. Similarly, Swamy et al. [72] reported strong antimicrobial and anti-inflammatory activity of Hexadecanoic acid. In addition, the *L. capsica* extracts were observed to have resistance to typical harmful bacteria such as *Escherichia coli* and *Salmonella typhimurium* expressed in immature composts made from livestock sludges [70]. Furthermore, Hexadecanoic acid displayed antibacterial activity against *Bacillus subtilis*, which is generally non-pathogenic and plays an important role beyond the middle phase of maturation during composting [44]. Based on the aforementioned results from a previous study, MB-derived hydrocarbon-based fatty acids serve to facilitate plant growth or can suppress harmful bacteria or pathogens in the soil environment as a positive biostimulant.

Although methods of catalysis, extraction, and degradation have been applied in various fields for securing MB, it is economically valuable to use the whole algae biomass. Thus, direct use of naturally dried MB as whole presents an economic advantage in large-scale agriculture. Although further study is warranted to elucidate the effect of useful ingredients in MB on the soil environment, economic viability, and aquatic environments, MB samples harvested from on-site coagulation/flocculation processes containing natural organic acids (e.g., humic/fulvic acid), various organic compounds and microorganisms can enrich the nutritional contents in soil environment to positively affect the composting or microbial fermentation processes leading to the enhanced production as bio-fertilizers.

### 3.6. Potential Application of Dried MB as Biostimulant

Considering a wide array of chemicals (i.e., Undecane; Heptadecane; Hexadecanoic acid, methyl ester; and Methyl stearate, phenolics, and fatty acids) extracted from MB samples in this study can be involved in signaling plant response to abiotic stress [65], plant growth of *Perilla* sp. with MB samples was monitored for 14 days. As displayed in Figure 7 and Table 5, plant growth and biomass with MB samples were greater than those without MB samples. These results indicate that MB was potentially beneficial due to the synergistic effects from combinations of MB-derived cyanobacteria and soils providing complementary behaviors (e.g., production of phytohormones, siderophores, N fixation, etc.). As summarized in Table 5, biomass productivity indicates that the increase in both fresh weight and leaves of *Perilla* sp. clearly depends on the applied dosage of MB. Especially for the experimental groups with greater amounts of MB, fresh weight, and number of leaves for *Perilla* sp. showed greater growth rate and no harmful effect. These results suggested MB contained bioactive green compounds derived from microalgae, and these green compounds could release the abiotic stress for certain plants through the enhanced bioavailability of macro and micronutrients.
Figure 7. Growth change of *Perilla* sp. by application of MB fertilizer. (a) control and (b) composted MB sample (37.6% w/w). I-IV shows the difference in biomass of *Perilla* sp. at each point by application of MB fertilizer.

Table 5. Biomass productivity results of *Perilla* sp. through the application of different MB fertilizers. A–d means a statistically significant difference for each analysis result.

| Experimental Groups              | Fresh Weight (g) | Leaves (num.) | Harmful Effects |
|----------------------------------|------------------|---------------|----------------|
| Control                          | 7.7 ± 0.33d      | 9.1 ± 0.59d   | None           |
| Composted sawdust                | 7.8 ± 0.40d      | 9.3 ± 0.51d   | None           |
| Composted MB sample (11.7% w/w)  | 8.2 ± 0.21d      | 10.0 ± 0.22d  | None           |
| Composted MB sample (21.6% w/w)  | 9.1 ± 0.35c      | 13.6 ± 0.68b  | None           |
| Composted MB sample (37.6% w/w)  | 11.3 ± 0.53a     | 15.9 ± 0.55a  | None           |

Similar results have been reported since the biostimulatory action of MB is attributed to both production and excretion of hormones (i.e., auxins and cytokinins) into the growing plants from the soil environment [73] and microalgal extracts can also mitigate the detrimental effects imposed on plant by the abiotic stress and drought [74]. Although there is general consensus on the potential benefits of the interaction between MB and plants, limited scientific evidence supporting this interaction has been provided, compared to other organic/inorganic biostimulants. Thus, further study is warranted to elucidate the complex interactions among microalgae, plant species, and soil environment, and to determine the optimal combinations (e.g., the weight ratio of MB to soil) in the agricultural field.

4. Conclusions

Surface characteristics, physicochemical properties, functional groups, and bioactive compounds of MB samples harvested from various WTFs in eutrophic water bodies were investigated to evaluate the reuse feasibility of MB as a potential renewable source of plant biostimulation. Since coagulation-flocculation processes in eutrophic water occurred through the interaction between positively charged salts and negatively charged matters, mixtures of the microalgal cells and fine particles (i.e., silt, clay, suspended solids, extracellular organic matter, humus substances, natural organic matter, etc.) were complexed inside MB samples. Considering that the surface of MB samples was found to be relatively rough and dense after drying without rupturing of MB cells from SEM images, MB samples harvested and air-dried under natural conditions applied in this study can have relatively well-preserved cellular morphology as well as chemical substances such as carbohydrates, proteins, and fatty acids. The EDS results displayed that the surface composition of C, O, Al, and Si was evenly distributed, and no significant difference in the elemental composition of the MB samples from different WTFs was found. The XRD results showed a broad form similar to the amorphous cellulose rather than a distinct crystalline. Although band assignment in FTIR results varies with position and intensity, there was a prominent peak at around 1088–1038 cm\(^{-1}\), indicating that the middle spectrum of glucose and starch hydrolysate exist in MB samples. A total of four hydrocarbon-based fatty acids compounds (Undecane; Heptadecane; Hexadecanoic acid, methyl ester; and Methyl stearate) were observed to be dominant and these compounds have been identified as green chemicals positively affecting germination, plant growth, and crop yield. Since a wide array of chemicals (i.e., Undecane; Heptadecane; Hexadecanoic acid, methyl ester; and Methyl stearate, phenolics, and fatty acids) extracted from MB samples were involved in signaling plant response to abiotic stress, plant growth and biomass with MB samples were greater than those without MB samples. These results indicate that MB was potentially beneficial due to the synergistic effects from combinations of MB-derived cyanobacteria and soils providing complementary behaviors (e.g., production of phytohormones, siderophores, N fixation, etc.). Thus, mixtures of nutrients, minerals and algal biomass in harvested and dried MB samples could be potentially reused in agricultural area as biostimulant after simple processes such as...
composting, microbial fermentation and extraction due to the presence of rich nutrients (macro and micro) and minerals. Further study is warranted to elucidate the effect of useful ingredients in MB harvested from on-site coagulation/flocculation processes on the soil environment to positively affect the composting or microbial fermentation processes as biostimulant and biofertilizer.

Author Contributions: Formal analysis, C.H.A.; Methodology, C.H.A.; Supervision, J.C.J. and J.R.P.; Writing—original draft, C.H.A.; Writing—review and editing, C.H.A., S.L., T.-M.H. and J.C.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Major Project of the Korea Institute of Civil Engineering and Building Technology, grant number 2020-0044 and 2020-0648.

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

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