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Flotation of microalgal particles without chemical coagulant and role of extracellular polymeric substances under auto-flocculation

Min-Ho Jang[a], Yong-Ho Choi[a], Yong-Hoon Jeong[a], Nguyen Thi Hong Hue[b], Dong-Heui Kwak[a,b,*]

[a]*Department of Bio-Convergence Science, Jeonbuk National University, 567 Baekjedae-ro, Deokjin-gu, Jeonju Jeonbuk 54896, Republic of Korea*
[b]*Department of Bio-Active Material Science, Jeonbuk National University, 567 Baekjedae-ro, Deokjin-gu, Jeonju Jeonbuk 54896, Republic of Korea, Tel. +82(0)63-530-7611; Fax: +82(0)63-530-7619; email: kwak124@jbnu.ac.kr (D.-H. Kwak)*

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ABSTRACT

To effectively separate and collect microalgal particles from a water body without using a chemical coagulant, a series of flotation separation experiments were conducted during the auto-flocculation life cycle phase addressing a problem in separated algal biomass recycling. Based on the analysis of extracellular polymeric substances (EPS), which participate in algae particle auto-flocculation, we investigated the role of EPS and evaluated its applicability as a chemical coagulant substitute in the flotation process. The sources of the main algal growth factors, carbon, nitrogen, phosphorus, and light intensity, were controlled to compare the differences in EPS components and separation efficiency among the cultivation samples. Comparative analysis of proteins and carbohydrates revealed no qualitative difference in the EPS affecting the auto-flocculation and flotation separation of particles. However, there was a slight difference in the quantitative EPS concentration by culture condition, and under high EPS carbohydrate concentration, a high separation efficiency was achieved under nutrient-deficient conditions. Moreover, the supply of CO_2 gas in a nutrient-deficient state promoted EPS production and increased the carbohydrate content, which induced algae particle hydrophobicity and promoted increased flotation efficiency. The study results suggested that microalgae particles can be separated in an eco-friendly manner using only EPS emitted by microalgae during auto-flocculation without a chemical coagulant during flotation.

Keywords: Algal biomass; Auto-aggregation; Extracellular polymeric substances; Flotation separation; Microalgae harvesting

1. Introduction

Excessive algal growth is known to cause problems such as secretion of toxic substances and off-flavor in a water body, interference with coagulation, and blockage of filtration in water treatment processes [1–3]. Various techniques such as filtration, centrifugation, and microbubble flotation have been applied to suppress the growth of algae or to remove flourished algal particles. Flotation has been reported as a useful technique in terms of the ecological and economical aspects of microalgal particle separation [4].

Flocculation is the most important factor in the flotation process for particle separation. Chemical coagulation using a coagulant is the typical method; however, there is a limit to recycling because of the coagulant component contained in the separated and recovered algal biomass. Although chemical coagulation is effective in removing low-density algae particles [5], it is sensitive to pH and may cause environmental problems due to the cost of coagulant and

* Corresponding author.

residual metal sludge [6]. Moreover, owing to the complicated coagulant injection facility, chemical coagulation is not recommended from an environmental and technical point of view. Therefore, it is necessary to develop a method to effectively remove algal particles without chemical treatment to replace chemical aggregation.

Challenges to aggregating and removing algae without chemical treatment have been widely attempted for many years. Auto-flocculation of microalgal particles by extracellular polymeric substances (EPS) is a separation method with many advantages because it does not require the addition of chemical coagulants and is environmentally friendly [7]. The auto-flocculation characteristics of algae appear mainly after the microalgae are sufficiently grown, that is, when EPS is produced [8]. EPS is a high-molecular substance composed of polysaccharides, proteins, hexanes, and lipids [9], and has a dominant effect on the auto-flocculation of cells and biological and inorganic substances owing to the interaction of polymer accumulate during the culture period [10]. The secreted EPS promotes the formation of agglomerates between microalgal particles and bonds cells close to the matrix [11]. Various culture conditions such as illuminance, nutrients, and pH can affect EPS production. In addition, the production and composition of EPS are significantly affected by the nutrient level, and the degree of auto-flocculation may vary depending on the protein or polysaccharide content inside [12].

Because the secretion of EPS is mainly affected by culture conditions such as nutrients, previous studies have been conducted to confirm the efficiency of auto-flocculation by controlling growth factors [13]. Glycine, an organic carbon and nutrient source, can affect biofilm formation and EPS secretion, and induce auto-flocculation of *Chlorella vulgaris* by controlling the amount of glycine. In case studies of *Chlorella* sp. and *Microactinium* sp., two types of microalgae produced abundant protein-rich EPS [14]. However, there was also a study that stimulated EPS production by *Thalassiosira pseudonana* under nitrogen-depleted conditions. In addition, Staats et al. [15] investigated the release of EPS through changes in the nitrogen to phosphorus (N/P) ratio according to microalgae species.

Although intense light and high temperatures are required, bicarbonate concentration and carbon can induce EPS generation in algal cells. It was revealed that the more carbon available, the more EPS was produced, and auto-flocculation of *Arthrospira platensis* was possible without the addition of a chemical coagulant [16]. EPS has a significant effect on auto-flocculation, and the separation efficiency is determined by the degree of aggregation of cell particles by EPS. Many studies have been conducted on precipitation by controlling the secretion of EPS in algal cells under cultured environmental conditions. However, because of the decomposition of precipitated algae particles, nutrients are re-emitted to the water body or precipitated to become hypnospores. Therefore, precipitation is not a fundamental measure for algae removal, because it germinates and proliferates again. Although studies have been conducted to examine the feasibility of flotation separation through auto-flocculation by controlling the EPS concentration, few studies have compared factors that change the concentration of EPS secretion depending on the actual EPS-causing nutrients and environmental conditions. Furthermore, no study has been conducted to separate or harvest microalgal particles from the water body through the application of auto-flocculation using EPS control in the flotation process.

To suppress eutrophication of lakes, we cultured algal samples under various conditions to separate algae particles without chemical coagulants using the conditions in which EPS is secreted, the algal particles can be aggregated spontaneously, and EPS can be analyzed. Accordingly, a series of flotation separation experiments was performed to efficiently separate microalgal particles through a bubble collector in the flotation process. The auto-flocculation of algae secretes EPS due to environmental stress caused by various factors, and the secreted EPS acts as a coagulant and cross-links the algae particles with each other. Therefore, the carbon source, illuminance, and nutrient concentration were adjusted to the culture conditions to find more favorable auto-agglomeration conditions for flotation separation. Based on flotation experiments, we attempted to determine the optimal conditions for auto-flocculation by EPS through a comparison of various environmental culture conditions.

2. Materials and methods

2.1. Microalgae cultivation

We selected *Chlorella vulgaris* as a freshwater microalga because it has a fast growth rate and responds quickly to conditions. Microalgae were cultured in the medium prepared by culturing the strain (AG10002) from the Center for Biotechnology and Biotechnology (Research Institute of Biotechnology, Korea), and the medium BG-11 (Sigma-Aldrich, USA) configured as shown in Table 1 was used. Before culturing microalgae in various environments, they were preliminarily cultured in a 15 L glass incubator to equalize the initial cell concentration and the environment of the culture medium. Cultivation conditions were maintained

Table 1
Media composition for microalgae cultivation during the initial cultivation phase and various experimental cultivation phases

Component	Concentration (mg/L)
Sodium nitrate	1,500
Potassium phosphate dibasic	40
Magnesium sulfate·$7H_2O$	75
Calcium chloride dihydrate	36
Citric acid	6
Ferric ammonium citrate	6
EDTA disodium magnesium	1
Sodium carbonate	20
Boric acid	28.6
Manganese chloride·$4H_2O$	18.1
Zinc sulfate·$7H_2O$	0.22
Sodium molybdate·$2H_2O$	3.9
Cupric sulfate·$5H_2O$	0.79
Cobalt nitrate·$6H_2O$	0.494

at 30,000 LUX and an average temperature of 25°C ± 1°C and cultured by continuous aeration for 5 d.

After the initial pre-culture, 15 L of microalgae was transferred to a 1 L Erlenmeyer flask for 17 d under the conditions listed in Table 2 to compare the EPS production and flotation separation efficiency according to the culture environment and further cultured. In each culture condition, K_2HPO_4 for phosphorus and $NaNO_3$ for nitrogen were dissolved in the culture medium and supplied, and carbon was periodically injected daily at 1,200 mL/h using a CO_2 gas cylinder. Algae changes during the incubation period were monitored continuously using the Chl-a concentration and pH. To determine the Chl-a concentration, microalgal samples were filtered using a 47 mm filter (Whatman, GF/C), and 10 mL of acetone (9 + 1) was added to the filter paper, followed by grinding. The ground samples were left at 4°C for 1 d, filtered, and measured using a UV Spectrophotometer (UV-160A, Shimadzu) at 630, 645, 663, and 750 nm for quantification. The pH was continuously measured using a pH meter (Orion, USA) to check the change depending on the algal growth and CO_2 injection.

2.2. Flotation experiments

A laboratory-scale batch-type DAF experiment apparatus was prepared for a series of microalgal particle flotation experiments. Fig. 1 shows a schematic diagram of the microalgae flotation separation experiment using the algae culture process and the DAF test apparatus. The saturator volume was approximately 0.67 L, and it was made of stainless steel to withstand pressure. The reaction column was 4 cm in diameter and 138 cm in height, and 0.8 L of cultured algae was injected into a circular acrylic column to perform a flotation separation experiment. Pressurized water (milky water) was injected through the lower part of the column so that microalgal particles and microbubbles could collide and combine smoothly. After injecting the pressurized water formed in a pressure device (5.0 atm) using an air bomb, a flotation separation time of 30 min was set to measure the flotation separation efficiency of the microalgal particles. The samples collected from the floating algal scum in the upper part of the reaction column were dried at 60°C for 24 h after filtering using a GF/C filter, and the separation efficiency was calculated based on the measurement results.

2.3. Measurements and analysis

EPS was extracted from microalgal samples cultured for 17 d. EPS is divided into bound-type (bound EPS) and dissolved type (soluble-EPS), depending on whether it is dissolved in water. Bound and soluble-EPS are closely and weakly coupled to the cell, respectively [17]. The structures of bound EPS are also classified into two types: tightly bound (TB)-EPS, which are tightly and stably bound to the cell surface, and loosely bound (LB)-EPS, which are loosely dispersed [18]. The EPS extraction method was used by applying a physical method with low extraction efficiency, but less interference in the analysis process than other methods. As shown in Fig. 3, the algal culture was centrifuged to extract soluble-EPS. Then, formamide was used to increase the extraction efficiency and reduce contamination by intracellular substances before the LB-EPS was extracted with the pellet [19]. After LB-EPS extraction, the recovered pellet was placed in distilled water, and ultrasonicated TB-EPS was extracted after treatment. All extracted EPS samples were separated using a 0.20 μm membrane filter.

Quantitative analysis was performed to investigate the physicochemical properties and changes in the EPS secreted under various culture conditions. To indirectly estimate the total amount of EPS, dissolved organic carbon (DOC) was measured with a total organic carbon (TOC) analyzer (multi-N/C, Korea) after filtration through a 47 mm GF/C filter. To reduce the OH peak interference without destroying the EPS extracted by the method shown in Fig. 2, the samples were dried at 60°C for 48 h to remove moisture. The dried sample was analyzed by Fourier-transform infrared spectroscopy (FT-IR; Frontier, Perkin Elmer) using the ATR (attenuated total reflection) method.

For EPS quantification, which may affect the separation efficiency of microalgal particles in the flotation experiments, carbohydrates and proteins, which are the major components of EPS, were measured quantitatively on the microalgal samples and scum after flotation. Proteins were measured according to the Lowry method [20,32], in which copper ions bind to the protein–peptide nitrogen and react under alkaline conditions. The anthrone method [21] was used to measure carbohydrate content by reacting with anthrone as a reduced sugar. Proteins and carbohydrates were quantified based on the measurement of absorbance at 750 and 620 nm using a UV Spectrophotometer (UV160A, Shimadzu,

Table 2
Cultivation conditions for the experiments of microalgae particles flotation

Samples	Growth factors		
	Nutrients	Carbon source	Light conditions
Initial cultivation phase	Nutrients supplied (with media, N:P 38:1)	CO_2 gas limited	15,000 Lux
AS-def/con (control group)	Nutrients limited (without media)	CO_2 gas limited	15,000 Lux
AS-def/C	Nutrients limited (without media)	CO_2 gas supplied (intermittent injection)	15,000 Lux
AS-def/ill	Nutrients limited (without media)	CO_2 gas limited	30,000 Lux
AS-sup/N	Nutrients supplied (with media) N:P 100:1 (N surplus)	CO_2 gas limited	15,000 Lux
AS-sup/P	Nutrients supplied (with media) N:P 1:1 (P surplus)	CO_2 gas limited	15,000 Lux
AS-sup/eqi	Nutrients supplied (with media, N:P 38:1)	CO_2 gas limited	15,000 Lux

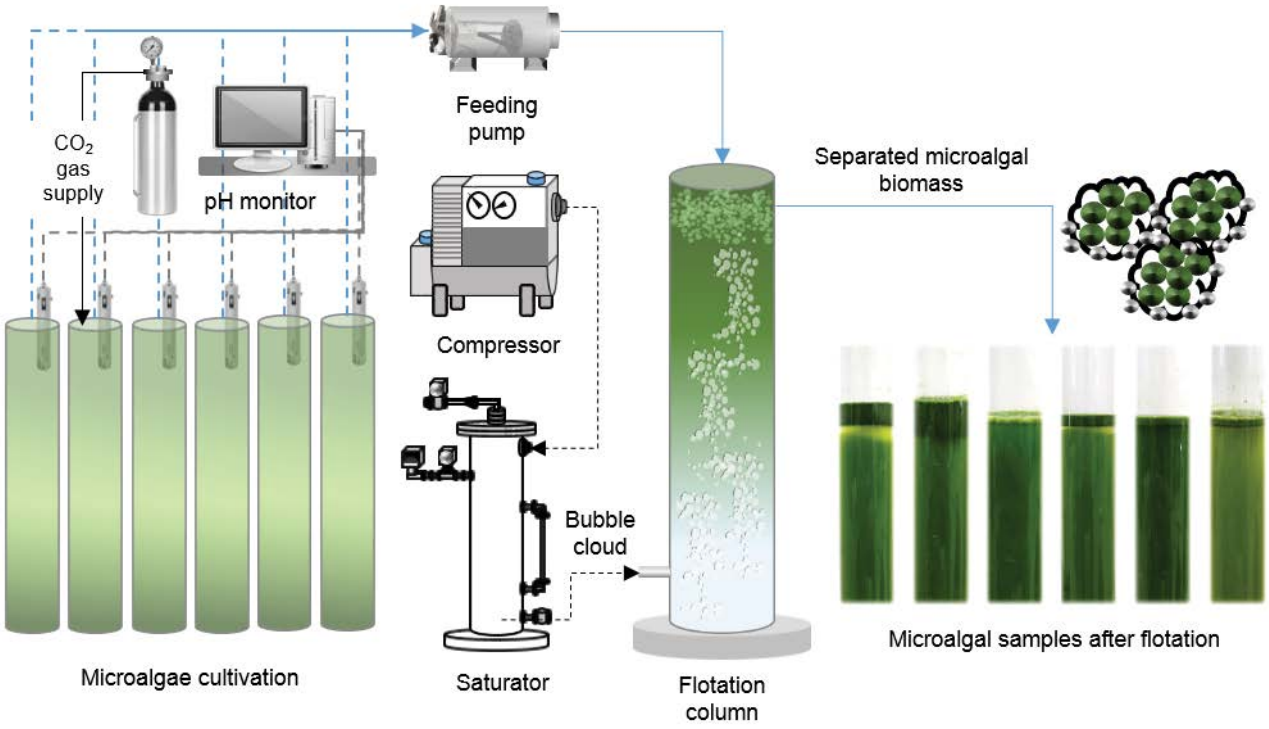

Fig. 1. Schematic diagram of microalgae particle separation process using batch-type flotation experiment apparatus.

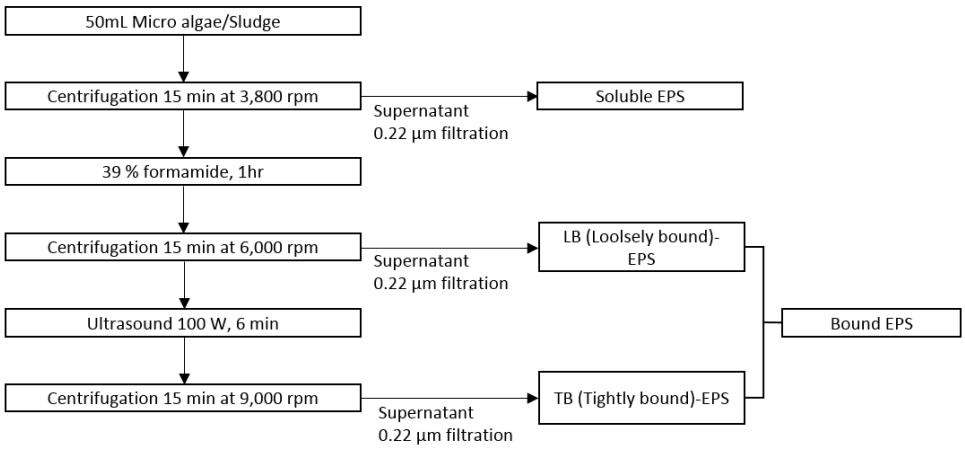

Fig. 2. Sequential extraction processes of EPS depending on the binding type of EPS onto microalgal cell.

Japan) by preparing a standard solution of bovine serum albumin (Sigma, USA) and lactose monohydrate.

3. Results and discussion

3.1. Growth characteristics under various cultivation conditions

EPS released from microalgal cells is affected not only by environmental cultivation conditions such as light, temperature, and incubation period but also by various factors such as the availability and concentration of nutrient sources [17]. The growth of microalgal cells is divided into growth, stationary, and endogenous phases. During the growth phase, algal cells have a high negative surface charge and are difficult to neutralize, so they are dispersed. When they reach the stationary phase, the negative charge becomes neutralized so that the cells aggregate to form a floc [22]. The life cycle of algae changes with cultivation and environmental conditions, which affect the auto-flocculation of microalgae. To determine the differences in the growth characteristics of microalgae under various cultivation environments and medium components, changes in pH and Chl-a were monitored continuously.

As shown in Fig. 3a, the algal cells seeded in the early stage of cultivation grew stably after going through a lag phase of approximately 2 d. After the initial 5 d, healthy

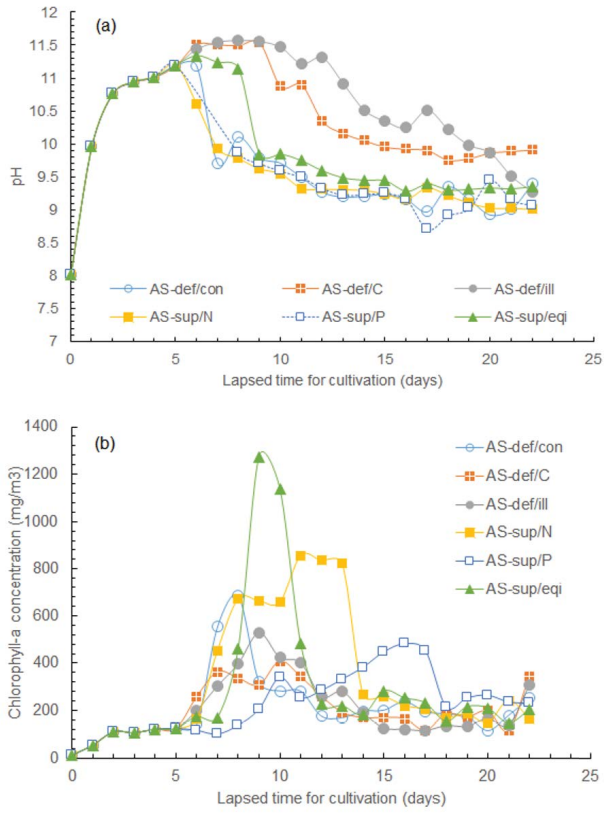

Fig. 3. Algae growth under various culture conditions in terms of cultivation time: (a) change in pH and (b) change in Chl-a.

strains were collected and cultured for 17 d according to the culture conditions shown in Table 2. Fig. 3b shows that the pH gradually decreased during the algae incubation period. In each culture condition, the pH value increased with the growth of algae, and after the algae grew to the maximum, Chl-a and pH gradually decreased. During the growth process of algae cells, daughter cells with their cell walls are formed inside the parent cell wall, and as the parent cell wall is ruptured, a part of the parent cell provides nutrients to the daughter cells, increases the number of daughter cells, and then gradually enters the endogenous phase [22]. The decrease in pH appears to be caused by the release of organic acids or cationic substances during cell death.

3.2. Components and characteristics of EPS

3.2.1. Functional group of EPS

The EPS emitted by microalgae is an important auto-aggregation material for algal cells and plays a role in coagulating algal particles without using chemical coagulants. EPS is known to provide a linkage mechanism among cells, organisms, and organic/inorganic compounds [23] and is affected by the availability of nutrients, including carbon sources and nitrogen, as well as culture conditions such as light, temperature, and incubation period [23]. In addition, EPS are known to have a difference in adhesion to cells depending on the dissolved or bound form [23].

The differences among the EPS samples taken under various cultural environment conditions were compared and examined to determine a more effective EPS configuration for auto-flocculation. Fig. 4 shows the FT-IR spectra of the six types of EPS samples obtained by dividing the EPS extracted from the microalgal culture samples into soluble-EPS and bound-EPS.

Qualitative analysis of EPS through the FT-IR spectrum showed almost the same peak shape as that of *Chlorella vulgaris* [24]. The main components of soluble-EPS shown in Fig. 4a are polysaccharides, proteins, nucleic acids, and lipids, and have various functional groups such as –COOH, –NH, –OH, and –CO– [25]. No notable differences were found between the samples extracted from the six culture conditions, and all had similar functional groups. In all samples, polysaccharide peaks due to hydroxyl groups appeared in the OH band at 3,320–3,455 cm^{-1}. Protein peaks of peptide carbonyl (amide I), amide II, and NH bands were analyzed at 1,646–1,649 cm^{-1}, 1,344–1,362 cm^{-1}, 1,238–1,245 cm^{-1}, 1,584–1,589 cm^{-1}, and 828–878 cm^{-1}. Carbohydrates and polysaccharides of the CO band and the α-glycosidic bond of the anomeric region at 878 cm^{-1} were identified [26]. In bound-EPS, as shown in Fig. 4b, the lipid functional groups of the CH and CC bands were confirmed at 2,716–2,941 cm^{-1}.

As described above, the presence of polysaccharides, proteins, and lipids was confirmed in the soluble-EPS extracted from the six samples. Similar to the soluble-EPS, no significant differences were found in bound-EPS. However, hydrophilic functional groups such as C–O and C–O–C were found in soluble-EPS, whereas polysaccharides, a hydrophobic functional group, appeared more clearly in bound-EPS.

3.2.2. Quantitative comparison of EPS

To quantitatively compare EPS analyzed in microalgal samples cultured under six different environmental conditions for 17 d, the DOC concentration and carbohydrate protein amount of each sample were measured. In general, it is known that the EPS production of microalgae increases, and the composition of EPS changes in an environment lacking nutrients [27]. As shown in Table 3, DOC concentration and total protein-carbohydrate content showed slight differences in the experimental results for each condition. The DOC concentration and total protein-carbohydrate values were high under nutrient-deficient conditions, AS-def/con (control group), and AS-def/C (CO_2 gas supplied). These results are considered to be the cause of the increase in EPS external emissions as the algal parent cells divide, and the cells grow and are deficient in nutrients. From the viewpoint of cell growth, EPS was found to be the same as in a previous study [28], which was detected mostly in the stationary or apoptotic phase.

In contrast, AS-def/ill, a nutrient-deficient condition but a strong light environment, did not show proper growth, and the protein-carbohydrate concentration of EPS was relatively low. Further studies on how light intensity affects EPS generation need to be conducted with more focus on the role of light in the future.

Comparing the concentrations of protein and carbohydrates in EPS analyzed in microalgae samples cultured under six different environmental conditions, AS-sup/P (N/P 1:1), which lacked phosphorus, had low protein and

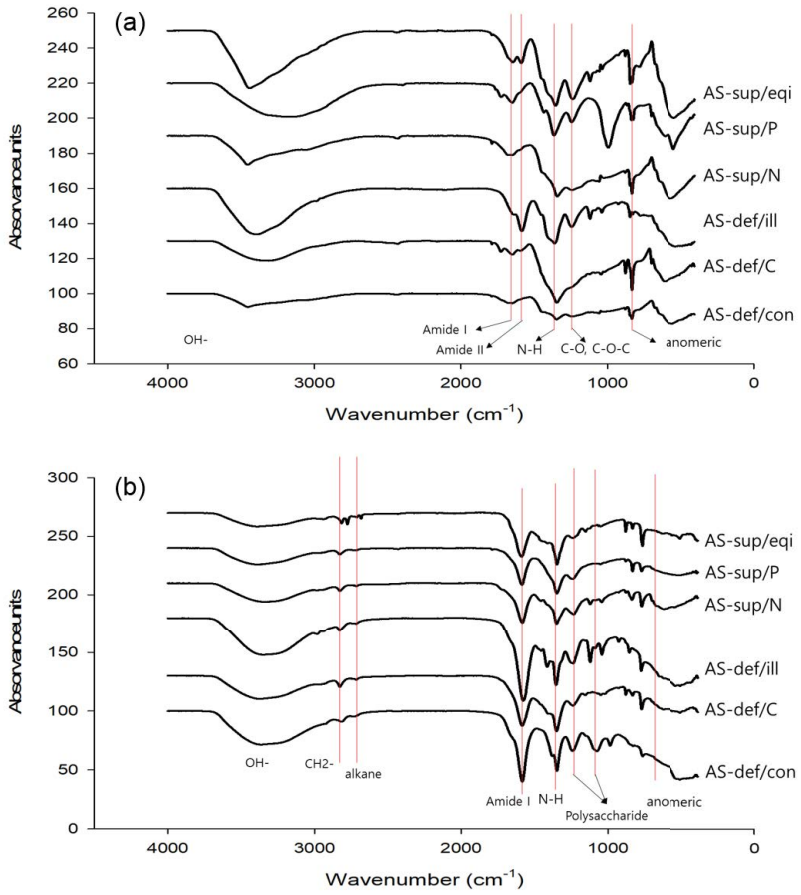

Fig. 4. FT-IR spectrum of six samples for various cultivation conditions: (a) soluble-EPS and (b) bound-EPS.

Table 3
Total EPS (protein, carbohydrate) and DOC concentration taken from microalgae cultivation samples under six types of environmental conditions

Samples	DOC (mg/L)	ECP (mg/L)		
		Sum (A + B)	Protein (A)	Carbohydrate (B)
Initial cultivation phase	14.27	–	–	–
AS-def/non	30.52	364.7	26.6	338.5
AS-def/C	33.12	366.3	26.7	339.5
AS-def/ill	24.64	287.4	23.3	261.1
AS-sup/P*	21.16	348.6	20.0	328.5
AS-sup/N*	17.50	310.0	37.4	272.5
AS-sup/eqi*	22.77	360.9	35.8	325.1

*Nutrients supplied after the initial lag phase.

carbohydrate content. Conversely, in AS-sup/N (N/P 100:1), which lacked nitrogen, the protein content was higher than that of the carbohydrates. Under nitrogen-deficient conditions, nitrogen acts as a growth-limiting factor, showing a high protein content in EPS, and under nitrogen-sufficient conditions, algae are considered to be the reason for excreting EPS outside of the cell rather than inside the cell [29]. In the nutrient-deficient AS-def/con (control group) and AS-def/C injected with CO_2 as a carbon source, the carbohydrate concentration of EPS was high, the protein concentration was somewhat low, and the protein concentration of EPS during the apoptosis stage showed the same result as in a previous study [30].

3.3. Auto-flocculation and flotation separation of microalgal particles

To effectively separate microalgal particles from water bodies without chemical coagulants, the separation efficiency was investigated in the flotation process. It is known that the flotation efficiency of microalgal particles is very low without a chemical coagulant dose because they do not aggregate owing to the homogeneous surface characteristics of algae particles, and the difference in charge with the bubble surface is very small [31]. To separate microalgal particles, well-aggregated flocs are formed through EPS secretion. EPS have a significant effect on agglomeration by inducing changes in the surface charge of microalgal particles [9]. EPS has various chemical structures and single or heteropolymer structures and is known to form a physical gel because of the interaction between proteins and carbohydrates [32,33].

The stable matrix structure of EPS forms a polymer network in which the EPS adheres to the surface through interactions [9]. Therefore, the degree of aggregation required for flotation separation formed by EPS was observed using an optical microscope.

Fig. 5 shows the difference in the degree of aggregation, which varies depending on the cultural environmental conditions and the cultural period. Comparing the culture periods of 12 and 17 d, it can be seen that the algae, which were dispersed throughout the 12 d of culture, were relatively aggregated during the culture of 17 d. In particular, the aggregation phenomenon of algae was more evident under conditions in which nutrients were not supplied, such as AS-def/con, AS-del/C, and AS-del/ill, than under conditions in which nutrients were supplied.

During the active growth of microalgae, the algal cell surface charge is low and negative, so the algal particles are dispersed, but it is known that the EPS released by the algal cell neutralizes the cells and forms agglomerates as it enters the stationary or endogenous phase [34]. Fig. 6 shows the results of particle separation of microalgae culture samples cultured for 17 d without a chemical coagulant dose, depending on the aggregation effect of EPS in DAF. The flotation separation experiment showed that the highest efficiency was achieved with a flotation efficiency of approximately 35% per cultivation cycle in AS-def/C (CO_2 injection condition). The flotation efficiency of the samples

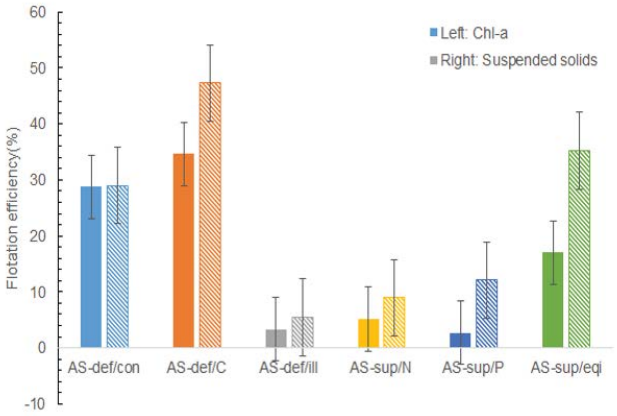

Fig. 6. Flotation efficiency of microalgae particles cultured for 17 d.

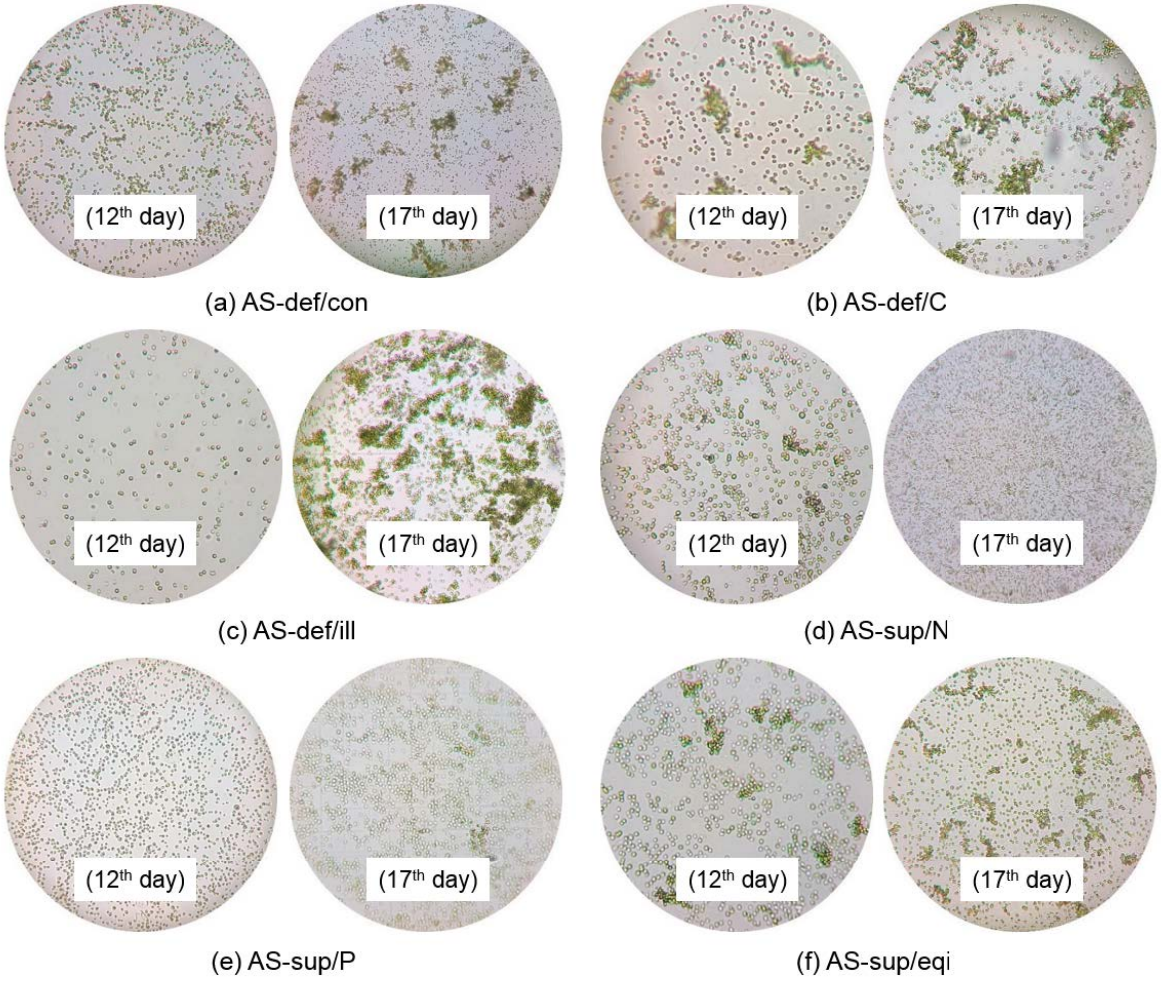

Fig. 5. Aggregation images of microalgae particles for six cultivation conditions captured by photomicroscope (400×).

cultured under nutrient-deficient environmental conditions was generally high, and the nutrient-supplied samples showed almost no flotation. These results show that, compared with the quantitative measurement results of EPS in Table 3, the efficiency of flotation separation was higher in samples cultured in nutrient-deficient environmental conditions, such as AS-def/con and AS-def/C, in which abundant EPS was released.

3.4. Evaluation of correlation

To examine the effect of EPS on the flotation of microalgal particles, the correlation between the culture medium composition and flotation efficiency according to the EPS properties was evaluated. Algal particles are negatively charged and are known to have hydrophobic properties. To form microalgal aggregates without chemical coagulants, aggregation mechanisms such as ion bridging through EPS and electrical double-layer compression are required [35]. The main components of EPS that affect auto-flocculation of algae particles are proteins, carbohydrates, and both components.

Fig. 7 shows the correlation between flotation efficiency and EPS components in the culture medium. As shown in Fig. 7a, in the analysis of the correlation between the flotation efficiency and the EPS concentration, the flotation efficiency increased as the carbohydrate concentration of bound-EPS increased, whereas the correlation between the carbohydrate concentration of soluble-EPS and flotation efficiency was not high. In addition, as a result of examining bound-EPS by dividing it into LB and TB types, as shown in Fig. 7b, we found no significant relationship with the binding type of EPS.

However, the flotation efficiency was found to have little correlation with both the solubility and binding form of the bound-EPS protein (Fig. 7c). According to a previous study [36], carbohydrates, such as polysaccharides, are hydrophobic in water, whereas proteins are hydrophilic.

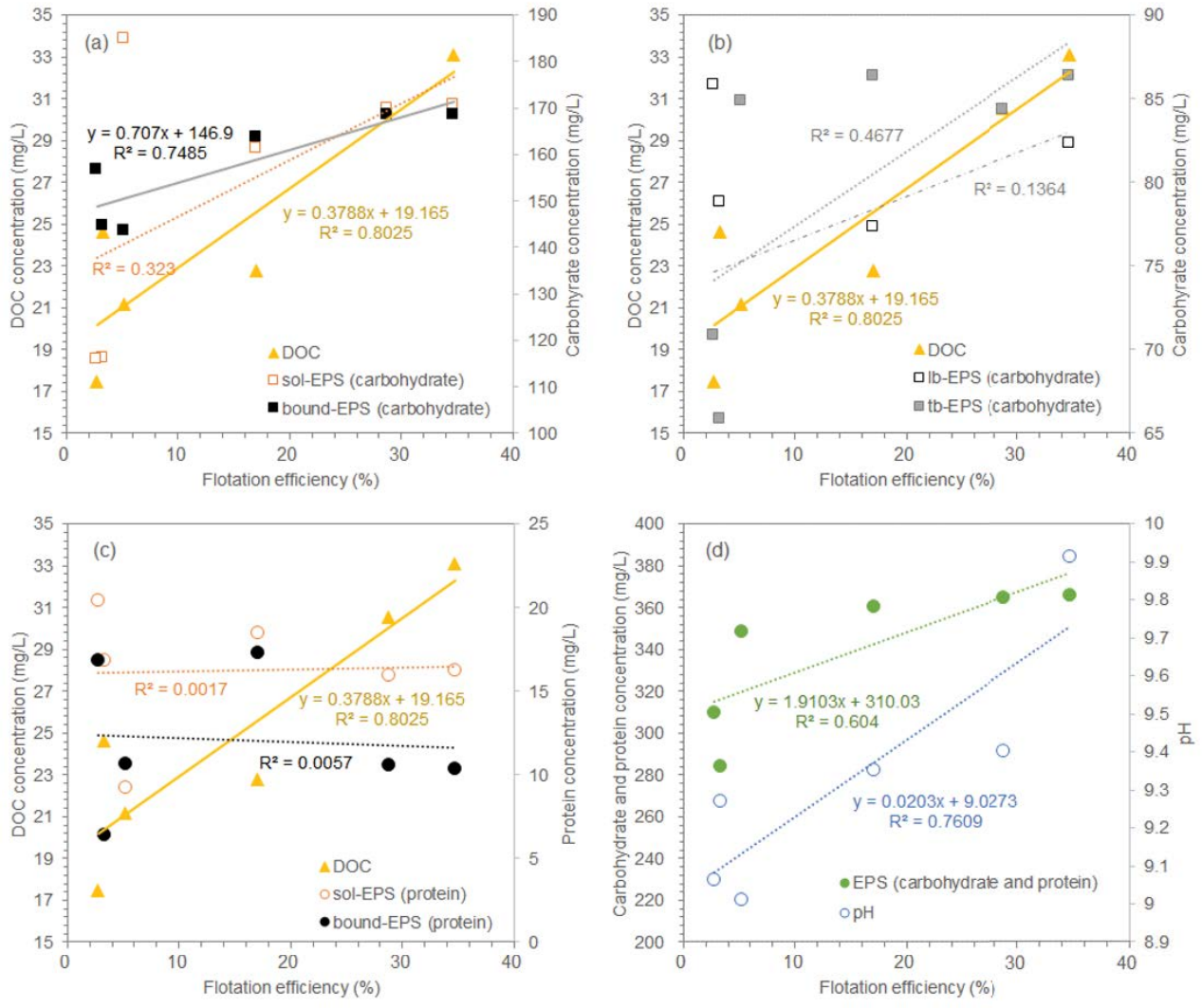

Fig. 7. Correlation between flotation efficiency and EPS types of cultivation media samples.

As shown in Table 3, the flotation efficiency was high in the AS-def/C sample, which was detected at a high carbohydrate concentration. This sample was cultured in a nutrient lacking an additional medium, and CO_2 gas was injected. The supply of CO_2 gas seems to promote EPS production and increase carbohydrate content. As the carbohydrate content increases, the algal particle hydrophobicity increases, which may lead to an increase in the particle flotation efficiency.

In addition, the flotation efficiency of algae particles was found to be correlated with the changes in the total carbohydrate-protein content and pH of EPS, as well as DOC, as shown in Fig. 7d. Correlation analysis revealed that the flotation separation of microalgal particles was related to the total amount of EPS discharged from algal cells. This finding led us to conclude that eco-friendly flotation separation of algae particles is possible by auto-flocculation through EPS optimization without a chemical coagulant.

4. Conclusions

The feasibility of microalgal particle separation was investigated by relying only on auto-flocculation secreted as a form of EPS by algal cells without chemical coagulants in the water body. EPS obtained from six types of samples under various cultivation conditions were analyzed qualitatively and quantitatively, and the flotation separation of algal particles was examined using a DAF experiment device. We drew the following conclusions from the observations and results of a series of experiments:

The main components of soluble-EPS are substances such as polysaccharides, proteins, nucleic acids, and lipids, and have various functional groups such as –COOH, –NH, –OH, and –CO. No special difference was found for each extraction sample under the six cultivation conditions, and similar functional groups were commonly identified in all samples. Similarly, in the bound-EPS, no qualitative difference was found in the six culture conditions. However, in the quantitative analysis of EPS, the DOC concentration and total amount of protein-carbohydrates showed differences for each condition. The DOC concentration and total protein-carbohydrate values were high under nutrient-deficient conditions, AS-def/con (control group), and AS-def/C (CO_2 gas supplied). In addition, in AS-def/C injected with CO_2 as a carbon source, the concentration of carbohydrate in EPS was high and the protein concentration was somewhat low, and the same result as in previous studies was shown in that the concentration of protein in EPS decreased during the endogenous stage. To observe the auto-flocculation required for flotation separation of microalgal particles, the phenomenon of algal cell aggregation in nutrient-deficient culture conditions rather than in nutrient-supplied conditions was observed through optical microscopy and macroscopy.

The highest separation efficiency was obtained from the flotation separation experiment, with a flotation efficiency of approximately 35% per cultivation cycle under CO_2 gas injection conditions. Compared with the quantitative measurement results of EPS, the flotation separation efficiency was relatively high in samples cultured under environmental conditions lacking nutrients, such as AS-def/con and AS-def/C, which produced abundant EPS. In contrast, the nutrient-supplied samples exhibited almost no flotation. As for the flotation efficiency, the correlation analysis with the EPS concentration showed that the separation efficiency increased as the carbohydrate concentration of bound-EPS increased, whereas the correlation between the carbohydrate concentration of soluble-EPS and flotation efficiency was not high. In addition, the protein of bound-EPS had little relationship with both the solubility and binding form. The supply of CO_2 gas in a nutrient-deficient state promoted the production of EPS and increases carbohydrate content. As the carbohydrate content increased, which increased the algae particle hydrophobicity, the microalgal particle flotation efficiency increased.

The flotation separation of microalgal particles is related to the total amount of EPS discharged from algal cells; to effectively remove algal particles from the water body, it induces the release of EPS to form auto-flocculation between particles without chemical coagulants. Based on the results of a series of flotation experiments and analyses, we concluded that flotation separation of microalgal particles is eco-friendly by auto-flocculation through EPS optimization without a chemical coagulant.

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