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Flotation of cyanobacterial particles without chemical coagulant under auto-flocculation

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Abstract. Although flotation techniques are often used for the removal of algal particles, the practicality of algae-harvesting technologies is limited owing to the complex and expensive facilities and equipment required for chemical coagulation. Here, we examined the feasibility of an approach to separating algal particles from water bodies without the need for chemical coagulants, depending on the condition of the algae, and to determine the optimal conditions. Using Anabaena sp., a cyanobacterium causes algal blooms in lakes, we stimulated auto-flocculation in algal particles without coagulants and conducted solid-liquid separation experiments of algal particles under various conditions. The six cultivation columns included in our analysis comprised four factors: Water temperature, light intensity, nutrients, and carbon source; auto-flocculation was induced under all treatments, with the exception of the treatment involving no limits to all factors, and algal particles were well-settled under all conditions for which auto-flocculation occurred. Meanwhile, flotation removal of auto-flocculated algal particles was attained only when nutrients were blocked after algae were grown in an optimal medium. However, no significant differences were detected between the functional groups of the extracellular polymeric substances (EPSs) of floated and settled algal particles in the FT-IR peak, which can cause attachment by collision with micro-bubbles.

Keywords: algal bloom; auto-flocculation; bubble; coagulation; cyanobacteria; flotation

1. Introduction

Reservoirs and dams have been and are being constructed in many countries around the world in order to secure water resources, with the abundance of water-retention areas greatly increasing as a result. Many of these water-retention areas are subject to blooms caused by excessive algae production, and therefore, control is an issue of growing concern in many countries. In many cases, the algal bloom of interest is caused and dominated by cyanobacteria, which are extremely difficult to remove (He *et al*. 2016).

Separation of algal particles by sedimentation is not effective on the control of the algal bloom owing to the decomposition products of algal sediments recycle repeatedly from the sediments of water body, more to the point, a permanent method is required to remove algal particles with the other process such as flotation, which can discharge algal particles from the water body of lake in filed. Separating algae from large bodies of water typically involves techniques that promote algal flotation, such as the dissolved air flotation (DAF) process (Laamanen *et al*. 2016, Naghdi and Schenk 2016). Although much research has focused on using bubbles to separate algae from water bodies (e.g., Henderson *et al*. 2010), this approach is not actively used in the field due to cumbersome equipment. On the other hand, the fundamental theories on the physiochemical and hydrodynamic interactions are well supported to attach bubbles on the surface of flocs in many studies including trajectory modeling (LaFrance and Grasso 1995, Edzwald 2010). However, the algal particles or bubbles are hardly controlled to attach each other dealing with two different phase material (solid, gas) in nature state. Most of all, most cumbersome operation practically is coagulant dosing in flotation process in field (e.g., algae harvesting ship).

The injection of chemical coagulants is especially problematic when the harvested algal biomass is intended for use as raw food material or for animal feed, owing to its potential toxicity; this is especially true for flotation processes, in which chemical coagulants are needed for the attachment of algae particles with bubbles (Borowitzka 1992). Moreover, additional steps to remove the coagulants are thus required, even when the harvested algal material is intended for bio-fuel production (Schenk *et al*. 2008). Finding a way to harvest algae under natural conditions, without the need for such steps as artificial pH adjustment or chemical coagulation, is thus key to improve the separation and use of algal particles.

Auto-flocculation, a process in which algae agglomerates into large masses, or flocs, during cultivation

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(González-Fernández and Ballesteros 2012), is often triggered in response to stressful environmental conditions, such as extreme pH levels, nutrient depletion, and changes in water temperature and dissolved oxygen content (Salim *et al*. 2011); the clumped algae is then highly desirable to solid-liquid separation. In addition, the extracellular polymeric substances (EPSs) that the algae secrete under stressful environment conditions affects flocculation between algal particles, as the primary function of the EPSs is to act as a physical barrier or protective film that prevents surface damage caused by desiccation and grazers (Sigee 2005). Secretion of EPSs occurs actively during starvation (Johnson *et al*. 2005). In the cultivation process, even the C:N ratio directly influences the electric charge and properties of the cell surface; when the C:N value increases, the surface electric charge also continuously increases (Shin *et al*. 2001). For instance, Córdoba-Castro *et al*. (2012) reported that EPS production by Scenedesmus obliquus increased in response to higher levels of CO2 in a culture medium, with both EPS output and algal growth/development maximized when CO2 was approximately 4% high. Conversely, EPS production decreased when CO2 levels were lowered.

Here, we conducted a solid-liquid flotation/ sedimentation separation experiment without chemical coagulants based on the stimulation of auto-flocculation of Anabaena sp., a cyanobacteria. The primary goals of this study were to determine the conditions under which auto-flocculation occurs and find out the suitable condition for particle separation of cyanobacteria in the flotation process.

2. Material and methods

2.1 Algae cultivation for auto-flocculation

Anabaena sp., a cyanobacteria commonly found in water-retention sites (Ahn *et al*. 2015), was used in this study, in the form of *Anabaena flos-aquae* (AG10026), which was artificially cultured indoors. The initial inoculum for the *Anabaena flos-aquae* culture was provided by the Korea Research Institute of Bioscience and Biotechnology.

BG-11 Medium for blue green algae (American Type Culture Collection Medium 616; Table 1) was used for the culture, with LED lamps serving as light sources instead of natural light. Open columns were used so that carbon could be supplied directly from the atmosphere, and CO_2 was supplied from a CO_2 pressure vessel in order to control the C:N ratio.

Flocculation of algae primarily occurs during the period of transition from the log growth phase to the plateau phase (Lavoie *et al*. 1984). Cell aging influences the formation of high-density cells as opposed to direct precipitation (Lavoie and de la Noüe 1987). According to several studies on algae-particle separation, separation efficiency is highest during the decline phase that follows the stationary phase in the DAF process (Jung *et al*. 2006, Zhang *et al*. 2012). Therefore, auto-flocculation can be induced by manipulating the four conditional factors within 10 days following primary cultivation regardless of the growth phase, as shown in Table 2.

Table 1 Composition of cyanobacteria growth medium (BG-11) for cultivation of *Anabaena* sp.

Nutrients	Trace metal mix A5
$NaNO_3$ 1.5 g	
K_2HPO_4 0.04 g	
$MgSO_4 \cdot 7H_2O$ 0.075 g	H_3BO_3 2.86 g
$CaCl_2 \cdot 2H_2O$ 0.036 g	$MnCl_2 \cdot 4H_2O$ 1.81 g
Citric acid 0.006 g	$ZnSO_4 \cdot 7H_2O$ 0.222 g
Ferric ammonium citrate 0.006 g	$NaMoO_4 \cdot 2H_2O$ 0.39 g
EDTA (disodium salt) 0.001 g	$CuSO_4 \cdot 5H_2O$ 0.079 g
Na_2CO_3 0.02 g	$Co(NO_3)_2 \cdot 6H_2O$ 49.4 mg
Trace metal mix A5 1.0 ml	Distilled water 1.0 L
Agar 10.0 g	
Distilled water 1.0 L	

Note: pH was 7.1 following sterilization

Table 2 Cultivation conditions for auto-flocculation, based on four factors

Cultivation (period)	Factors for auto-flocculation formation			
	Water temp.[a]	Light intensity[b]	Nutrients source[c]	Carbon source[d] (C:N ratio[e])
Primary culture (10d) Subsequent culture	25 °C	27 μmol m^{-2} s^{-1}	Supplied	Limited (20±1)
Run-A (10d)	18 °C	27 μmol m^{-2} s^{-1}	Supplied	CO2 supplied*(>20)
Run-B (10d)	18 °C	Limited	Supplied	Limited(<20)
Run-C (10d)	28 °C	27 μmol m^{-2} s^{-1}	Limited	Limited (>20)
Run-D (10d)	28 °C	27 μmol m^{-2} s^{-1}	Supplied	CO2 supplied*(>20)
Run-E (10d)	33 °C	27 μmol m^{-2} s^{-1}	Supplied	Limited (<20)
Run-F (10d)	33 °C	27 μmol m^{-2} s^{-1}	Limited	Limited (>20)

*CO_2 gas was to supply carbon source from pressure vessel. a: Lupi *et al*. 1991, Moreno *et al*. 1998, b: Moreno *et al*. 1998, Rebolloso-Fuentes *et al*. 1999, c: Lee *et al*. 2009, d: Cordoba-Castro *et al*. 2012, e: Xia *et al*. 2008, Ntsaluba *et al*. 2011.

For the multi-factor experiment design based on the operation range of factors affecting on the algae growth in natural state, water temperature, light intensity, nutrient source, and carbon source constitute the four main factors that influence the growth of algae and stimulate auto-flocculation in lake. Algae were cultivated under different conditions, with the goal of inducing auto-flocculation. The solid-liquid separation experiment was conducted by flotation and sedimentation at 3-day intervals, respectively, during which algae particles were observed closely in order to ascertain whether or not auto-flocculation is time-dependent.

2.2 Separation of cyanobacterial particles

A batch type of separation columns was used in order to compare the separation characteristics of algae particles in

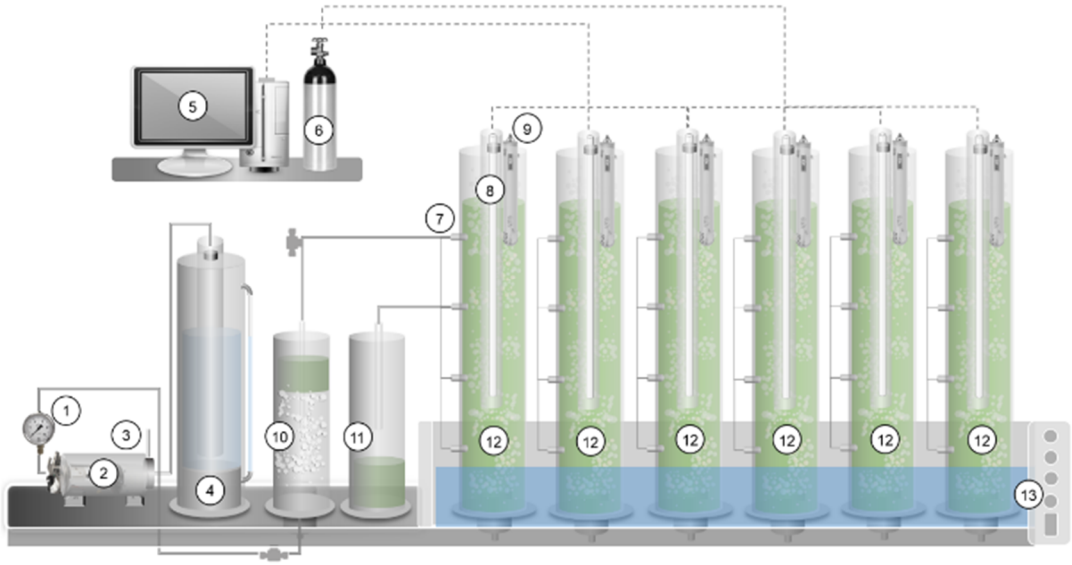


Fig. 1 Schematic diagram of cyanobacteria cultivation and the algal-particle separation apparatus. Note: 1. Flow rate gauge, 2. Milky water supplier, 3. Ejector, 4. Water tank, 5. Monitoring computer, 6. CO_2 pressure vessel, 7. Sampling port, 8. LED lamp, 9. Additional heater, 10. Flotation column, 11. Sedimentation column, 12. Algae cultivation column (A~F), 13. Water bath

Table 3 Dimension of DAF apparatus and equipment

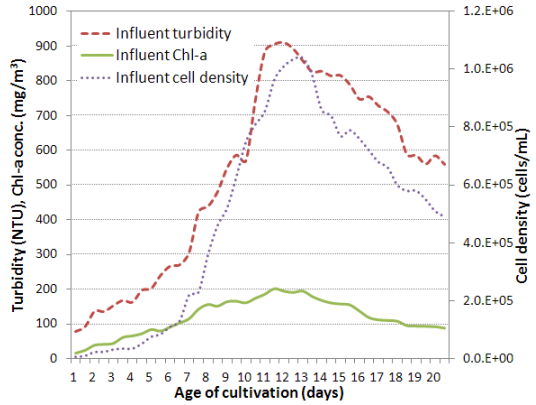
Compressor
Inflow air, 111 L/min, Outflow air, 60 L/min
Operation range, 8 atm (maximum)
Saturator
Diameter, 148 mm, Height, 430 mm, Total volume 7.40 L
Mass flow controller
Maximum pressure, 9.9 kgf/cm2
Control range, 0-10 kgf/cm2
Air flow rate, 0-10 L/min
Flotation column
Diameter, 100 mm, Height 1,000 mm
Total volume, 7.85 L (effective volume 6.28 L)

culture solution between flotation and sedimentation, as shown in Fig. 1. The algae grow in the batch type of cultivation columns (Run A to F) setting up in the water bath. Several units such as CO_2 pressure vessel, sampling ports, LED lamps, and additional heaters, are attached in the cultivation columns. The samples collected from the sampling ports in the column are examined for the sedimentation in the column and for the flotation in the column. Other apparatus, flow rate gauge, milky water supplier, ejector, and water tank, are required for the flotation in the column. The diameter of all the columns (cultivation, flotation and sedimentation) made of plexiglass was 10 cm, and the height of the cultivation and the separation (flotation and sedimentation) columns was 50 cm and 30 cm, respectively.

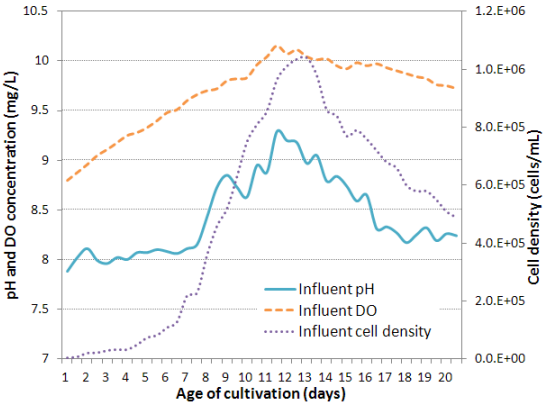
Algae particles taken from each cultivation column were suspended initially in the flotation and sedimentation columns, then bubbles were introduced in the flotation column from the bottom side of the column. After all the bubbles in the cell reached the top of the column, the solution was sampled to obtain the flotation efficiency. Micro-bubbles (average diameter 38 μm) from an ejector pump bubble-generating apparatus were injected to act as collectors of algae particles in the flotation column; amount of milky (oversaturated) water containing micro-bubbles was supplied to levels as 10% of the target sample, with a contact time between bubbles and particles of 1 min and a separation time of bubble-floc agglomerates of 10 min (Kwak *et al*. 2009, Kim and Kwak 2014). In the sedimentation experiment, separation efficiency was evaluated by analyzing the supernatant after allowing it to settle for 30 min.

In the laboratory scale plant dimensions for the DAF shown in Table 1, air bubbles were applied for attachment on the surface of flocs formed by auto-flocculation. The saturator pressure was from 405.3 to 607.95 kPa in the DAF. A pressure gauge was installed at the top of the saturator to ensure the correct pressure.

Significant differences between cultivation conditions were determined by two-way ANOVA and post-hoc Tukey tests (= 0.05). A two way analysis of variance was conducted to clarify the significant ($p < 0.05$) in algal particle separation between flotation and sedimentation as well as between the six cultivation conditions of Anabaena sp. The two way ANOVA analysis of chlorophyll-a and cell density measurements showed notable difference between flotation and sedimentation ($F = 3.208$, $p < 0.05$). In general, the particle separation efficiency of sedimentation did not differed significantly between the five cultivation conditions except one condition whereas in flotation separation, the only one condition showed high efficiency ($p < 0.01$).

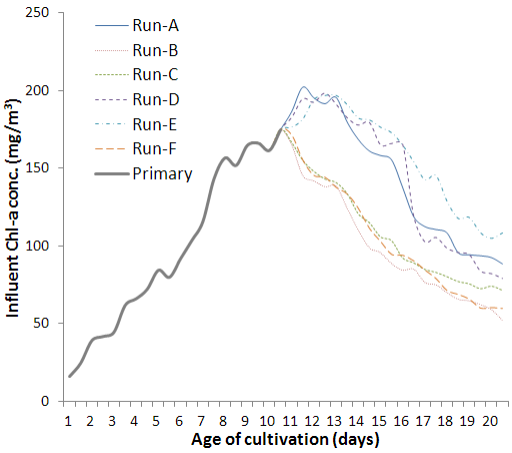


(a) Algae particles

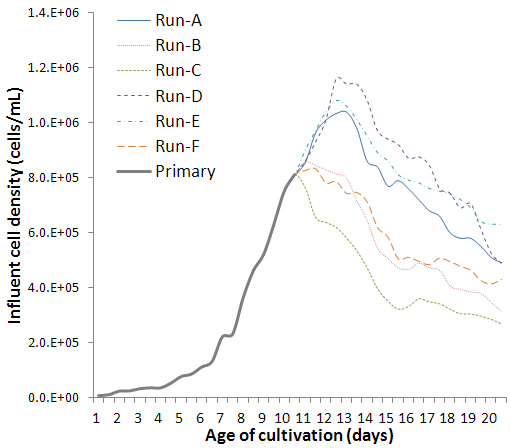


(b) Culture solution

Fig. 2 Variation of algal particles and conditions in Run-A in terms of culture age for Anabaena sp. cultivation period



(a) Chlorophyll-a



(b) Cell density

Fig. 3 Variation and comparison of algal particles in terms of culture age for Anabaena sp. cultivation period

The water quality was measured by the standard methods for water and wastewater (APHA 2005). The chlorophyll-a concentration was measured using the UV-VIS spectrophotometer (UVmini-1240, SHIMADZU), and the functional group of algal compounds was analyzed to identify the difference of EPSs using Fourier Transform Infrared Spectrometer (FT-IR; Frontier, Perkin Elmer).

3. Results and discussion

3.1 Variation of cultivation

The functional group on the algal surface determines protonation, so that its influence on algae harvesting depends on the pH of the culture medium and is related to photosynthetic efficiency (Wyatt *et al*. 2012). However, because the pH of most natural waters in a range of about 6 to 8.5 due to chemical interactions among carbon dioxide, hydrogen ions, and the anions that produce alkalinity buffer, pH was not adjusted artificially in the experiments. As a result of continuous monitoring, the average and range of pH for Run-A was 8.44 and 7.88-9.29 for the cultivation period, as shown in Fig. 2. The pH of the algae culture solution was not high enough to trigger auto-flocculation in the algae.

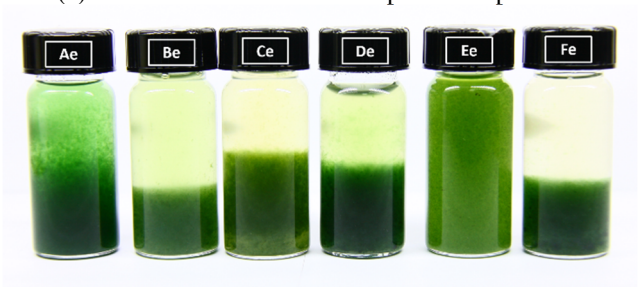
It is known that oxygen produced via photosynthesis controls the electrostatic repulsion and van der Waals forces of EPS (Barsky *et al*. 1984), and thus large and compact flocs are often formed in response to high oxygen concentrations (Wilén and Balmér 1999). As the cultivation columns were open, oxygen transfer and dissolved oxygen (DO) concentration were also measured continuously. The DO of Run-A was 9.68 mg/L on average and ranged between 8.80 mg/L and 10.15 mg/L, and the average dissolved concentration is 123.8% of the saturation level at 28°C.

Fig. 3 represents the variation of chlorophyll-a and algae cell density for Run-A to Run-F in the cultivation periods following primary cultivation, in which variations in algae growth patterns were observed: Algae populations in Run-A, Run-D, and Run-E grew continuously for 2-3 days and then began to gradually decline, whereas populations declined immediately in Run-B, Run-C, and Run-F.

3.2 Auto-flocculation

The appearance of the algae cultivation solution compared with the various cultivation conditions is shown in Fig. 4(a). There was an obvious distinction among the samples taken on the 5th day of subsequent cultures for Run-A to Run-F. Differences in color and algal flocs were

(a) Cultivation solution before particle separation



(b) Cultivation solution after clarification

Fig. 4 Algal-particle flocculation and conditions in column Run-A ~ Run-F

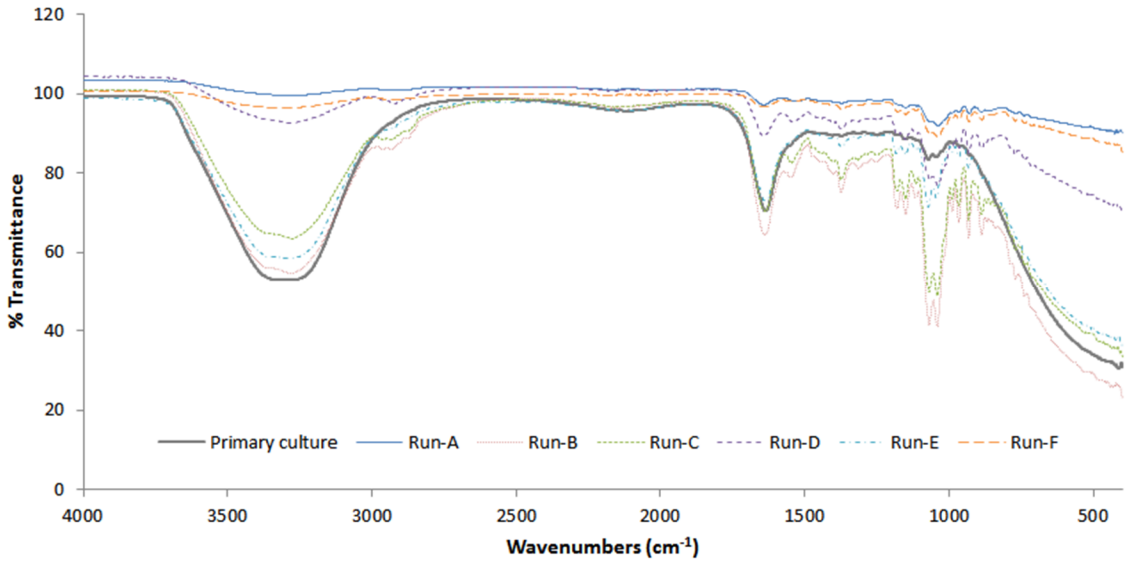


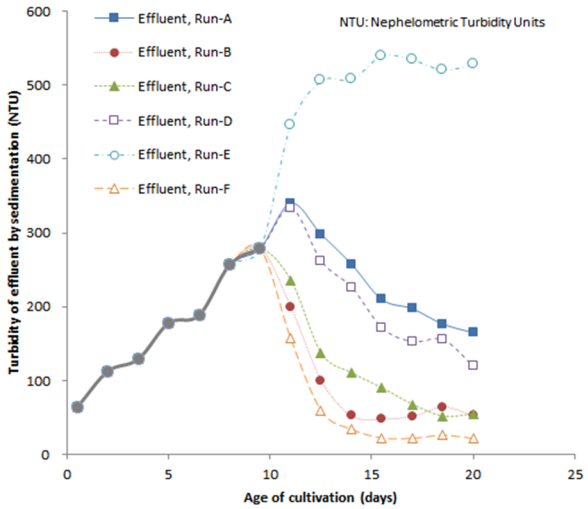
Fig. 5 FT-IR spectrum of algal particles (Run-A through Run-F).

observed; the color of the Run-A and Run-D samples, which were provided with CO_2, was a dark green, with some algal floc formations observed, whereas very few algal flocs were observed for Run-E, in which algae were cultured under temperatures as high as 33°C, although it presented a dark green color. On the other hand, the Run-B and Run-C samples were light green in color.

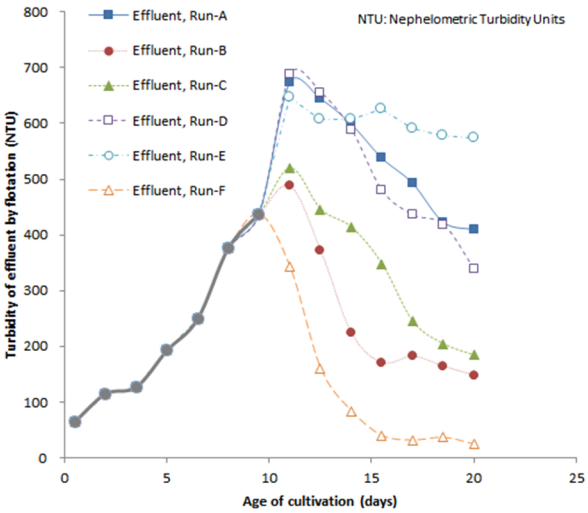
Characteristics of the samples following sedimentation are shown in Fig. 4(b). This figure highlights the markedly different ways in which algae responded to the various cultivation conditions. Although the algal flocs of Run-A and Run-D (CO_2 supplemented) settled a little, the supernatants were colored a faint green due to the presence of residual particles, and flocs exhibited a tendency toward sedimentation in Run-C and Run-F (nutrient limited) and Run-B (reduced light intensity). The supernatant of Run-F (nutrient limited, high temperature) was the clearest of all treatments; however, no sedimentation was observed for Run-E (no limiting factors, high temperature).

Cell aging stimulates the secretion of EPSs in batch cultivation of algae (Zhang *et al*. 2012). In addition, cyanobacteria flocculation depends on the composition and chemical structure of the EPSs rather than the volume of the EPS secretion (Andreadakis 1993). Given the influence of the EPSs on the auto-flocculation and tendency toward sedimentation of algal flocs, an FT-IR spectral analysis was performed to determine the composition and functional group of the EPS produced by this alga species. The FT-IR revealed that the main component of the EPS was commonly carbohydrate (Fig. 5). The slow peak and wide

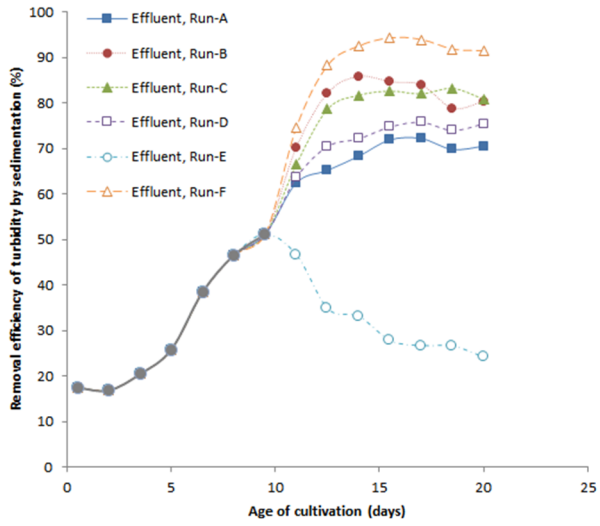
curve at 3,000-3,800 cm–1 in the spectrum represents H_2O, which is very often observed in wet samples due to residual water contents; the principal component was found to be a polysaccharide, represented by the gentle curve at 3,000-3,600 cm–1, the peak at 1,032 cm–1 (attributed to the O–H bond), and a peak at 1,100 cm–1 (attributed to the single C–O bond). These results accord with previous studies concluding that EPSs are composed primarily of carbohydrates and contain little protein (Sigee 2005, Dugdale *et al*. 2006). However, little differences in the functional groups were found among the six spectra of the FT-IR analysis in terms of the various cultivation conditions.



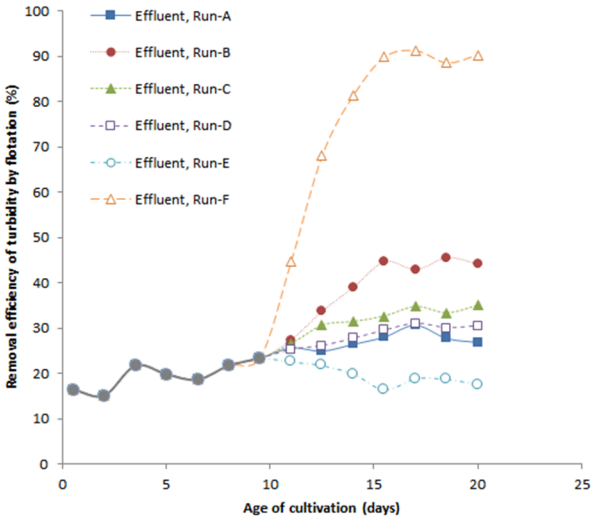
(a) Turbidity of sedimentation



(b) Turbidity of flotation



(c) Separation efficiency of sedimentation



(d) Separation efficiency of flotation

Fig. 6 Variation of algal-particle separation efficiencies in terms of culture age for *Anabaena* sp. cultivation period

3.3 Particle separation efficiency

Sedimentation velocity was a very slow 10^{-5} to 10^{-6} m/s (Schnoor and Di Toro 1980, Granados 2012) because sedimentation separation depends on the density of algal particles similar to that of water. Changes in turbidity and chlorophyll-a concentrations during the cultivation period are shown in Fig. 6, with the turbidity of the supernatant following sedimentation and flotation presented in Fig. 6(a) and Fig. 6(b), respectively. After 12 days, over 70% of chlorophyll-a was removed except Run-A to Run-E, about 60% in Run-A, and less than 40% in Run-E removed via sedimentation, as shown in Fig. 6(c). Separation efficiency of chlorophyll-a showed a tendency toward gradual decline in accordance with the elapsed time of cultivation. The efficiency of algae-particle sedimentation was high in samples where auto-flocculation was observed. These results represent that auto-flocculated algal particles used to well settle and emit decomposition products affecting on eutrophication from the sediments on bottom of the lake in field.

Flotation efficiency differed greatly from the sedimentation efficiency (Fig. 6(d)). Separation efficiency was high in the Run-F sample (nutrient limited, high temperature), at approximately 90%, whereas flotation efficiency was low in all other samples, including Run-B and Run-C. Thus, our experiments demonstrated that adequate separation efficiencies by flotation could not be

achieved in all samples in which auto-flocculation occurred, and that auto-flocculation is a necessary condition for improving flotation efficiency.

Here, we have shown that inducing the auto-flocculation via nutrient restriction at optimal growth temperatures can be an effective strategy for separating particles of the algae *Anabaena* sp., a process for which the application of chemical coagulants is not necessary for biomass recovery. Currently, the application is an unrealistic approach for lake/reservoir remediation by controlling the temperature or limiting nutrients but needs to keep studying for avoiding the sedimentation of algae particles and removing or separating algae particle from the water bodies and then not repeating bloom outbreaks. Therefore, this study is worthy for biomass recovery and also needs more efforts for lake/reservoir remediation such as nutrient deficiency throughout the water quality (non-point pollution) management.

The meaningful finding of this manuscript is the feasibility that flotation without coagulant can be applied in field. However, the factors affecting the attractive interaction between algal particles and bubbles as well as auto-flocculation cannot be controlled in the nature state of lake. Based on the results of this study, further study is needed to find out when the auto-flocculation of algal particles take place and how to form the specific optimal condition of flotation in field.

4. Conclusions

A cyanobacterium was cultivated under various conditions for the purpose of inducing auto-flocculation to separate algal particles from the water bodies, and a series of solid-liquid separation experiments was conducted to examine the feasibility of separation without chemical coagulation using flotation over the entire cultivation period. *Anabaena flos-aquae* were cultivated under six different environmental conditions comprised of four factors – water temperature, light intensity, nutrient source, and carbon source. Auto-flocculation was induced under all conditions except one (high temperature, nutrients supplied, and low C:N ratio), and all algal particles taken from auto-flocculated culture solutions were well-settled in the sedimentation column. However, effective separation by flotation was achieved only under the conditions of nutrient limitation and high water temperature.

On the one hand, the differences between the functional groups of the EPSs of floated and settled algal particles were not detectable by FT-IR analysis. Regardless, the feasibility of algae particle separation (one species of cyanobacteria, *Anabaena flos-aquae*) via nutrient restriction and high temperatures, with no need for chemical coagulants, was confirmed in this study. Further research is required to identify optimal control solutions for separating the algae particles via flotation under the field conditions.

Acknowledgments

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