Flotation of algae for water reuse and biomass production: role of zeta potential and surfactant to separate algal particles
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ABSTRACT
The effect of chemical coagulation and biological auto-flocculation relative to zeta potential was examined to compare flotation and sedimentation separation processes for algae harvesting. Experiments revealed that microalgae separation is related to auto-flocculation of Anabaena spp. and requires chemical coagulation for the whole period of microalgae cultivation. In addition, microalgae separation characteristics which are associated with surfactants demonstrated optimal microalgae cultivation time and separation efficiency of dissolved CO2 flotation (DCF) as an alternative to dissolved air flotation (DAF). Microalgae were significantly separated in response to anionic surfactant rather than cationic surfactant as a function of bubble size and zeta potential. DAF and DCF both showed slightly efficient flotation; however, application of anionic surfactant was required when using DCF.

INTRODUCTION
Global climate change and energy issues are very serious, and many countries are developing fossil-fuel alternative energy sources to control greenhouse gas emissions. Recently, algae have received attention as a useful biomass resource to produce environmentally friendly bio-fuels. In producing microalgae as a biomass, CO2 gas is used for cultivation and flotation is applied for harvesting (Uduman et al. 2010; González-Fernández et al. 2012). Also, algal blooms are known as a seasonal environmental problem in water bodies such as lakes and reservoirs worldwide (Yung et al. 1999; Anderson et al. 2002; Yin et al. 2011).

Microalgae bio-fuel production has been extensively researched, and recent studies have found that economic and energetic issues need to be overcome (Stephens et al. 2010; Davis et al. 2011; Gonzalez-Fernandez et al. 2012). Many studies (Golueke & Oswald 1970; Sukenik & Shelef 1984; Edzwald 1995; Henderson et al. 2008; Davis et al. 2011) examined algal characteristics for water treatment and evaluated the impact of the varying properties and the use of pre-processes for coagulation and flocculation on their removal by solid-liquid separation processes. The process is strongly affected by the condition of microalgae harvesting. Generally, microalgae rapidly accumulates various organic substances using growth factors such as a light and CO2. Many microalgae are unicellular microorganisms and barely settle because they have a similar density to water, negative surface charge, and low settling velocities ($10^{-5}$–$10^{-6}$ m s$^{-1}$) (Sigee 2005; Henderson et al. 2008). The low settling velocities are strongly affected by intrinsic features such as extracellular polymeric substances (EPSs), which are macromolecular compounds that are excreted by the cell or released during lysis and promote biological auto-flocculation.

Harvesting algae and algae removal are different but operate on the same principles. Hence, to find a suitable technique which has to meet the needs of both processes, experts have developed several techniques including centrifugation, flocculation, filtration, sedimentation, and flotation. Uduman et al. (2010) reported that compared with other techniques, centrifugation consumes a large amount of electricity and may cause cell damage because of high shear forces. Coagulation is typically induced by addition of chemicals. The cheapest technique may be filtration, which is carried out using different membranes and depends on microalgae characteristics such as size and morphology. However, harvesting unicellular small...
cells is difficult. The main advantage of filtration is that cells are entirely preserved; however, they may be damaged while being pumped towards the screen (Vonshak & Richmond 1988). Conventional gravity sedimentation (CGS) removes between 70 and 80% of cells, while dissolved air flotation (DAF) tends to have more efficient removal rates, with a removal of more than 90% of the cells.

Lately, DAF has become much more popular as an effective solid/liquid separation or algae removal process for the low density of algae (Okada et al. 1990; Edzwald 1995). Flo-}

tation is the most effective and economic technique for harvesting cultivated microalgae. Microalgae were previously harvested from wastewater treatment by flotation to separate microalgae from water; the harvested or floated microalgae were a biomass resource and the treated water could be reused. DAF was the most robust clarification method, with up to 90% removal or harvesting during the water treatment process. Recently, dissolved CO₂ flotation (DCF) has been used as an alternative DAF to decrease CO₂ emission and to supply carbon source in microalgae cultivation (Kwak & Kim 2013). However, successful clarification heavily relied on preceding coagulation and flocculation optimization. If microalgae biomass production is applied for food or feed, chemical coagulants may not be useful because of their potential toxicity (Borowitzka 1992).

Chemical coagulation is used for optimum separation of microalgae. Henderson et al. (2009) compared 60–95% separation efficiencies which were due to three different chemicals: metal coagulant, cationic surfactant, and cationic polymer. However, chemical coagulation use might not be ideal because of the potential toxicity of chemical coagulant if algae biomass is used for food or feed (Borowitzka 1992). Thus, coagulant dosage should be reduced while biological auto-flocculation is required as much as possible for microalgae harvesting.

Microalgae auto-flocculation was first introduced in the 1970s by Gouleeke & Oswald (1970), and Sukenik & Shelef (1984) identified auto-flocculation due to co-precipitation with ions at a high pH, release of EPSs, release inhibition of daughter cells, and microalgae–bacteria interaction. An auto-flocculation mechanism with precipitation of magnesium hydroxide is proposed to explain a sweeping flotation of Dunaliella salina cells related to no effect on the recovery efficiency by the flow rate of sodium hydroxide addition, which reduced the concentration factor only for abrupt injections (Besson & Guiraud 2013). Besson & Guiraud (2013) examined the high-pH-induced flocculation of the hypersaline microalgae Dunaliella salina by sodium hydroxide addition. In addition, auto-flocculation of microalgae in cultivation is dependent on illumination, temperature, oxygen, and nutrient deficiency, which is related to EPS production. In particular, microalgae aggregation is affected by O₂ which is produced by photosynthesis to control attractive and repulsive forces between EPS (Barsky et al. 1984), and high O₂ concentrations may form large and compact flocs (Wilén & Balmér 1999). The major stress factor which influences EPS production is nutrient deficiency (Lee et al. 2009). In addition, CO₂ that is involved in photosynthesis is the main nutrient which affects flocculation because carbon is assimilated 6.5 and 100 times more than N and P, respectively (Sukenik & Shelef 1984). EPS production increased when CO₂ was added to the culture broth, which indicates that it is involved in microalgae growth. Aging of microalgae cultivated in batch model resulted in increasing extracellular polymers but the settling of microalgae was instead attributed to the increased cell density attained upon culture aging (Zhang et al. 2012). Little settling and negative surface charge of the microalgae was obviously associated with functional groups of the cell wall. For examples, zeta potential as an indicator of cell stability declined from the exponential to stationary microalgal growth phase of Chlorella zofingiensis accompanied with surface functional groups (Zhang et al. 2012).

This study determined separation characteristics of Ana-
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bara spp. which are associated with air and CO₂ bubbles and evaluated optimal operation conditions and the algal particle separation efficiency of the flotation process. Removal efficiency of chlorophyll-a was compared for the separation processes of algal particles, CGS, DAF, and DCF. Consequently, the laboratory experiments focused on flocculation control for chemical coagulation and biological auto-flocculation of Anabaena spp. in terms of the zeta potential of algal particles and cultivation time. Chemical coagulation produces high separation efficiency, which is good for reusing water, while biological auto-flocculation results in an eco-friendly microalgae harvesting for producing pure biomass. In addition, microalgae separation characteristics which are associated with surfactants demonstrated optimal microalgae cultivation time and separation efficiency of DCF as an alternative to DAF.

MATERIALS AND METHODS

Comparative experiments on algal particle separation were conducted under chemical coagulation and biological auto-flocculation. For the experiments, Anabaena spp. was obtained from the Korean Collection for Type Cultures.
For cultivation at the initiatory stage for 7 days, a 1L-Erlenmeyer flask equipped with a gauze stopper and autoclave was used for 50 mL of *Anabaena* spp. (blue-green freshwater algae), and 500 mL of BG11 (blue-green) medium. The regular cultivation in photo-bioreactor was conducted for 15 days with stirring at a rate of approximately 190–250 rpm at 30 °C in a pH range of approximately 7.4–8.4. To conduct algal particle separation experiments, different ages of algal samples were gradually collected from photo-bioreactor during the cultivation periods, and the separation efficiencies and characteristics of algal particles using three types of processes (CGS, DAF, and DCF) were examined.

As shown in Figure 1, a laboratory-scale batchwise reactor was designed to conduct a series of experiments to separate algal particles by micro-bubbles. The size of the circular acrylic batch reactor was 7 cm in diameter and 1.5 L in volume. The saturator (pressure tank) for DAF and DCF was operated at 4.0–6.0 atm and 1.5–2.1 atm, respectively, and a pressure gauge was attached to the upper saturator in order to apply the correct pressure. Air or CO2 gas was injected into the saturator through the mass flow controller and dissolved in recycled water. The recycled water with dissolved air or dissolved CO2 from the saturator and algal culture from the photo-reactor was injected into the flotation column in order to separate algae and liquid by DAF or DCF.

Zeta potential and chlorophyll-a were selected to investigate the physico-chemical characteristics of algal particles based on pH, cultivation time, and experimental conditions. For the zeta potential measurement, algal particles were measured using a Photal Otsuka ELS-8000 while bubbles were analyzed by an image analyzer equipment. The equipment consisted of a measuring cell, video camera, and image analyzing software. The method is based on electrophoresis, which was modified from an instrument from a previous study (Okada & Akagi 1987). Zeta potential was measured to investigate the surface charge to obtain an additional insight into the mechanism of particle separation, and measurement of chlorophyll-a concentration was conducted to evaluate algal particle separation efficiency. Conversely, chemical products used for chemical coagulation included poly-aluminum chloride (PAC) as inorganic coagulant and sodium hydroxide (NaOH) as alkalinity supplements. To examine the separation of algal particles (*Anabaena* spp.’s cells), chemical coagulation was conducted using 200–500 ppm of PAC and 0–100 ppm of sodium hydroxide.

This study used three different surfactants, including cationic cetyltrimethyl-ammonium bromide (CTAB, CH₃(CH₂)₃N(CH₃)₃Br, CAS no. 8001-54-5), anionic sodium lauryl sulfate (SLS, NaC₁₂H₂₅SO₄, CAS No. 151-21-3), and non-ionic polyoxyethylene glycol octylphenol ethers (Triton X-100, C₁₄H₂₉O(C₂H₄O)ₙ (n = 9–10), CAS no. 9002-93-1), to examine the change in zeta potential according to the surfactant type. Each surfactant used 2 mL per 100 mL of sample.

**RESULTS AND DISCUSSION**

**Zeta potential of bubbles and algal particles**

Henderson *et al.* (2008) revealed that coagulation and flocculation are very important for sedimentation and flotation. Particularly in the flotation process, the zeta potential of particles is one of the main parameters which make the positive particles converted by chemical coagulation attach to the surface of negative bubbles. Henderson *et al.* (2008) determined that zeta potential between −8 and +2 mV was optimal for algal particle separation.

The zeta potential of air and CO₂ bubbles was positive when the pH was about 3 but decreased to a negative value when the pH was greater than 4. The zeta potential of bubbles was strongly negative from −31.25 to −36.88 mV if the pH was above 7.0. The bubble zeta potential did not show any noticeable difference between air bubbles of DAF and CO₂ bubbles of DCF in a pH range of 3–11, as shown in Figure 2. Both kinds of bubbles showed typical zeta potential values in the operation range of their own saturator pressure. However, there were some changes in the zeta potential measurements of algal particles depending on two factors: (1) effect of pH on zeta potential for one suspension of algal particles; and (2) effect of cultivation time on the evaluation of zeta potential (Figure 3). As a
common acknowledged fact, the zeta potential of algal particle curve in terms of pH became more negative with increasing pH, which was artificially adjusted using sulphuric acid and sodium hydroxide as shown in Figure 3(a). The pH of the photo-bioreactor is gradually increased by photosynthesis of algae as the cultivation time elapsed. Instead of drawing a scatter graph using all of the raw data (50 data sets every culturing day), the mean value is plotted for the zeta potential of algal particles for the 15 culturing days in Figure 3(b). In the graph the mean values are used, and the error bars represent the standard error. The error bars based on the standard error (s.e.m.) statistically describe uncertainty in the mean and its dependency on the sample size, \( n = 50 \) (s.e.m. = s.d. / \( \sqrt{n} \)). When making more measurements, the standard error becomes smaller and it reflects the greater confidence in the mean values.

**Chemical coagulation and auto-flocculation**

To address coagulation and flocculation of microalgae, the algal particle separation experiments were conducted to compare separation efficiency using CGS, DAF, and DCF processes. When *Anabaena* spp. grew during cultivation in a photo-bioreactor, turbidity increased with cultivation time in a pH range of approximately 7.65–9.6. The turbidity changed from 177 to 695 NTU over 15 days.

To investigate effective separation conditions, chlorophyll-a was measured under the chemical coagulation in terms of cultivation time. Figure 4(a) shows the separation efficiency of chlorophyll-a by CGS, DAF, and DCF. After 7 days, DAF produced slightly better results than CGS, and its separation efficiency of chlorophyll-a reached 83.7–87.4%, while DCF which produced macro-bubbles of CO\(_2\), showed comparatively low separation efficiency compared to DAF because the macro-bubbles had less chance to be attached to the algal particles (*Kim & Kwak 2014*). The separation efficiency of chlorophyll-a of DAF was improved slightly 5.0–8.5%
compared with that of CGS as auto-flocculation was observed after day 7 as shown in Figure 4(a).

Better algae separation may be possible with an enhanced understanding of algal physiology and characteristics (Zhang et al. 2014; Besson & Guiraud 2013). However, research regarding auto-flocculation is interesting because chemical coagulation may not be useful when algae biomass is to be used for food or feed because of the potential toxicity, as mentioned in a previous study (Borowitzka 1992). Algal particle separation experiments were conducted to determine whether or not algae harvesting without chemical coagulation is feasible. As shown in Figure 4(b), algal particle separation efficiency was not sufficient for application in the DAF and DCF separation processes. Based on the experimental results, although chemical coagulation is still required to gain high efficiency compared with auto-flocculation, auto-flocculation demonstrated the possibility of reaching the high algal removal efficiency if the physico-chemical properties of algal particles are improved for microalgaee separation.

**Application of surfactants**

Surfactants can be used to improve the physico-chemical properties of particle surface as bubble surface modifiers in the flotation process (Somasundaran & Ramachandran 1988; Henderson et al. 2009). Comparative experiments were carried out to improve the separation efficiency of flotation with and without anionic surfactant (SLS) during chemical coagulation and auto-flocculation. As shown in Figure 5(a), there was a relatively larger increase of separation efficiency in DCF than DAF. In Figure 5(a), the best results of the DCF process with PAC only or with PAC and SLS were observed on day 9. The reason for comparing only the results of day 9 in Figure 5(b) was because good auto-flocculation was observed on day 9. Consequently, to enhance the separation efficiency of the DCF process relative to surfactant type, three different surfactants were used for flotation experiments. Anionic surfactant (SLS) yielded better results than the other two types of surfactants (CTAB and Triton X-100), and the difference of separation efficiency of chlorophyll-a was 14.6–16.0% when comparing sodium lauryl sulfate and none as marked in Figure 5(b). According to the experiment results, use of a PAC and anionic surfactant worked better than the use of PAC alone.

Finally, the zeta potential and rise velocity of bubbles were measured using Photal Otsuka ELS-8000 and the image analyzer equipment, respectively, to investigate the cause of separation efficiency enhancement by the addition of surfactant. Figure 6(a) shows that zeta potential depends on the types of surfactants, and there was no significant change of zeta potential by the addition of surfactant. The surfactants used in this study had no significant effect on the zeta potential (at the 0.05 level, $p$-value = 0.84), and were found to enhance the flotation efficiency by reducing the bubble size generated in the saturator. The change of zeta potential was less than 5 mV except when using only an anionic surfactant at a pH over 10, whereas the rise velocity of the CO$_2$ bubble cloud dramatically changed and

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**Figure 4** | Comparison of algal particle separation efficiency with and without chemical coagulation under auto-flocculation; saturator pressure: DAF 5.0 atm and DCF 1.7 atm, bubble volume concentration: DAF 278 mL/L and DCF 511 mL/L, average bubble size: DAF 46 μm and DCF 79 μm, and PAC dose: 500 ppm for both DAF and DCF (Kim & Kwak 2014b).
was different based on surfactant type, as shown in Figure 6(b). When a surfactant was not used, the mean rise velocity was fast (19.4–28.3 cm/sec). For the cationic surfactant, the mean rise velocity decreased by approximately 9.0 cm/sec, and for the non-ionic surfactant, by approximately 1.3 cm/sec, and for the anionic surfactant, by approximately 0.7 cm/sec. When the anionic surfactant was added, the rise velocity of the bottom layer was greatly decreased from 10.71–0.27 cm/sec at the effective mean saturator pressure 1.7 atm (1.5–2.1 atm for DCF). The measurement results of zeta potential and rise velocity reveal the rise velocity difference of the CO₂ bubble cloud; by the use of the surfactant, the reduction of CO₂ bubble size produced much better results compared with the change of zeta potential.

**CONCLUSIONS**

This study determined the effect of chemical coagulation, and biological auto-flocculation relative to zeta potential
was examined to compare flotation and sedimentation separation processes for algae harvesting. In addition, microalgae separation characteristics which are associated with surfactants demonstrated optimal microalgae cultivation time for flotation and separation efficiency of DCF as an alternative to DAF. A series of algal particle separation experiments were carried out, and the findings were as follows:

1. The bubble zeta potential did not show any noticeable difference between air bubbles and CO₂ bubbles.

2. A noticeable zeta potential increase of algal particles was observed from the day 9, which is when some green algal agglomerates began to be observed with the naked eye in the photo-bioreactor. At the same time, the separation efficiency of algal particles significantly improved with the zeta potential increase after entering the auto-flocculation period.

3. Chemical coagulation is required to gain high efficiency (over 40%) even during auto-flocculation if the physico-chemical properties of algal particles are not improved for microalgae separation.

4. Of the three types of surfactants, the anionic surfactant made the rise velocity of bubbles slower and yielded much better results than the other two types of surfactants. Thus, to apply CO₂ bubbles for microalgae harvesting, an anionic surfactant is useful to improve the bubbles.

In summary, this study determined that the separation characteristics of *Anabaena* spp. occurred as a result of chemical coagulation–biological auto-flocculation and depended upon zeta potential. If the algal biomass is used for food or feed, chemical coagulation may not be good and auto-flocculation may be ideal for pure microalgae harvesting. However, the physico-chemical properties of bubbles or algal particles must further be improved during auto-flocculation.

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REFERENCES


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