Flocculation Effect of Alkaline Electrolyzed Water (AEW) on Harvesting of Marine Microalga *Tetraselmis* sp.

Su-Jin Lee¹, Woo-Seok Choi¹, Gun-Hoo Park¹, Tae-Ho Kim¹, Chulhong Oh¹,², Soo-Jin Heo¹,², and Do-Hyung Kang¹,²*

¹Jeju International Marine Science Research & Education Center, Korea Institute of Ocean Science & Technology, Jeju Special Self-Governing Province 63349, Republic of Korea
²Department of Marine Biology, Korea University of Science and Technology, Jeju Special Self-Governing Province 63349, Republic of Korea

Microalgae hold promise as a renewable energy source for the production of biofuel, as they can convert light energy into chemical energy through photosynthesis. However, cost-efficient harvest of microalgae remains a major challenge to commercial-scale algal biofuel production. We first investigated the potential of electrolytic water as a flocculant for harvesting *Tetraselmis* sp. Alkaline electrolyzed water (AEW) is produced at the cathode through water electrolysis. It contains mineral ions such as Na⁺, K⁺, Ca²⁺, and Mg²⁺ that can act as flocculants. The flocculation activity with AEW was evaluated via culture density, AEW concentration, medium pH, settling time, and ionic strength analyses. The flocculation efficiency was 88.7% at 20% AEW (pH 8, 10 min) with a biomass concentration of 2 g/l. The initial biomass concentration and medium pH had significant influences on the flocculation activity of AEW. A viability test of flocculated microalgal cells was conducted using Evans blue stain, and the cells appeared intact. Furthermore, the growth rate of *Tetraselmis* sp. in recycled flocculation medium was similar to the growth rate in fresh F/2 medium. Our results suggested that AEW flocculation could be a very useful and affordable methodology for fresh biomass harvesting with environmentally friendly easy operation in part of the algal biofuel production process.

**Keywords:** Marine microalgae, harvesting, flocculation, alkaline electrolyzed water, *Tetraselmis* sp.

Introduction

Microalgae, which are photosynthetic microorganisms, are capable of transforming light energy into chemical energy and contain numerous bioactive compounds. They have been widely used in various industries for the production of food, feed, cosmetics, nutraceuticals, and pharmaceuticals [1–4] and are a promising renewable the energy source for biofuel production [5]. However, commercial production of microalgal biodiesel is not yet economically viable [6]. The process of microalgal biodiesel production consists of cultivation, harvesting, lipid extraction, and transesterification. The harvesting of microalgae is one of the major bottlenecks of industrial-scale biofuel production [4]. This stage usually accounts for 20–30% of the total production cost of biomass, which is 50% of the total cost of biodiesel production [1, 3]. The harvesting process is energy intensive and expensive owing to the small size of algal cells (1–30 µm), negative cell surface charge, and low cell densities in the culture medium (0.5–2 g/l) [1, 7]. Therefore, it is necessary to develop a harvesting technology that can treat a large scale of microalgae in a cost-efficient and environmentally friendly manner. To overcome these above problems, many researchers have suggested various harvesting methods, such as gravity sedimentation, filtration, centrifugation, flocculation, and dissolved gas flotation [5, 8].

The flocculation method is more easy to operate and suitable for large-scale utilization compared with other harvesting methods [4]. However, several disadvantages have been identified regarding flocculation. In the case of an inorganic flocculant, it may affect downstream processes or the quality of the algal biomass [1], whereas biodegradable...
organic flocculants are too expensive for use in low value-added products such as biodiesel and are not suitable for harvest of marine microalgae owing to the high ionic strength of seawater [7, 9–12]. Bioflocculation requires cost owing to the initial culture of microorganisms and separation of flocculants from the microorganisms [13, 14]. Microbiological contamination of the biomass can interfere with the application of microalga biomass for use as food or feed [15]. Physical flocculation using ultrasound is not suitable for large-scale harvesting of microalgae because of its high energy consumption and cost [16].

Electroflocculation, which is environmentally friendly and cost effective, has been studied as a method for harvesting microalgae. Moreover, this method has been reported to be more efficient for harvesting marine microalgae owing to the high ionic strength of seawater. However, the disadvantage of this method is the cost of electricity and the requirement of regular replacement of the sacrificial anode owing to fouling [4, 7, 17, 18]. Electroflocculation is an electrochemical method that induces flocculation of microalgae by metal ions such as Al³⁺ and Fe³⁺ released from the anode by electrolytic oxidation [7].

Alkaline electrolyzed water (AEW) can be considered as an alternative to electroflocculation for harvesting microalgae since it has mineral ions that can act as cationic flocculants. It is generated at the cathode by electrolysis of water containing electrolytes such as sodium chloride. AEW is characterized by a high pH (11–13.8), low oxidation–reduction potential (ORP; –750 to –900 mV), high dissolved hydrogen concentration, and positively charged mineral ions such as Na⁺, K⁺, Ca²⁺, Fe⁷⁺, and Mg²⁺ [19]. The use of AEW is more cost-effective than direct electrolysis of microalgae because it uses less electricity and does not require electrode replacement. In addition, AEW is inexpensive, easy to use, and environmentally friendly [20, 21].

Although there have been various studies of methods for harvesting microalgae, the effect of AEW on the flocculation of microalgae has never been reported. Therefore, the purpose of this study was to evaluate the potential of AEW for flocculation of the marine microalga Tetraselmis sp. The effects of flocculant dosage, sedimentation time, pH, and cell density on the flocculation efficiency were investigated. The viability of flocculated cells and recycling of flocculation medium were also examined.

Materials and Methods

Algal Strain and Cell Cultivation

*Tetraselmis* sp. was obtained from Inha University (Korea). The microalga was cultivated in 2-L Erlenmeyer flasks containing F/2 medium in seawater with continuous aeration [22]. The cultures were grown at 25°C under 12 h light, 12 h dark cycles and light intensity of 75 ± 5 µmol·m⁻²·s⁻¹. Growth rates of *Tetraselmis* sp. were monitored by measuring the optical density (OD) at 680 nm with a spectrophotometer (OPTIZEN POP; Mecasys, Korea).

Preparation of AEW

AEW was purchased as a commercial product (Aqua Nax, Korea), which is produced by electrolysis of 0.1% K₂CO₃ solution. The pH and ORP of AEW were measured using a dual scale pH/ORP meter (Fisher Scientific, USA). Mineral concentrations in AEW were determined using inductively coupled plasma mass spectrometry (Agilent Technologies, Japan). The pH, ORP, and mineral contents of AEW are shown in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>12.3</td>
</tr>
<tr>
<td>ORP (mV)</td>
<td>–450</td>
</tr>
<tr>
<td>Alkalinity (mg/l)</td>
<td>1,078</td>
</tr>
<tr>
<td>Sodium (mg/l)</td>
<td>57.2</td>
</tr>
<tr>
<td>Potassium (mg/l)</td>
<td>649</td>
</tr>
<tr>
<td>Magnesium (mg/l)</td>
<td>ND</td>
</tr>
<tr>
<td>Calcium (mg/l)</td>
<td>ND</td>
</tr>
<tr>
<td>Iron (mg/l)</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not determined; ORP, oxidation–reduction potential.

Determination of Microalga Biomass

The biomass concentration of the microalga was evaluated on the basis of the OD and dry cell weight. The OD was measured using a spectrophotometer at a wavelength of 680 nm. The dry cell weight was determined by filtration. Algal cells were filtered onto...
a pre-weighed GF/C filter (Whatman, USA). The filters were dried at 55°C for 24 h and weighed after cooling to room temperature. The dry cell weight was expressed in grams per liter.

Cell Viability
Alga cell viability was evaluated by the Evans blue staining method [23]. The alga culture (1 ml) was centrifuged at 990 x g for 5 min, and the supernatant was discarded. The cell pellet was stained with 100 μl of 1% Evans blue solution for 2 h at room temperature. Then, the cells were washed with phosphate-buffered saline and observed by light microscopy (TE2000-U; Nikon, Japan). Evans blue does not enter intact cells, but penetrates the membrane of damaged or dead cells and stains them blue [24].

Recycling of the Flocculation Medium
Microalgae flocculation medium that had been treated with 20% AEW was centrifuged at 3,000 rpm for 10 min. The supernatant was collected to test recycling. The pH of the flocculation medium was adjusted to 8.0 with 0.1 M NaOH, and F/2 medium was added. Control medium was fresh F/2 medium. Tetraselmis sp. was cultivated at 25°C for 12 days in both media. Cell growth was monitored as the absorbance at 680 nm using a spectrophotometer.

Results and Discussion

Effects of AEW Dose and Settling Time on the Flocculation Efficiency
The flocculation efficiency of Tetraselmis sp. was investigated using different concentrations of AEW and sedimentation time (Fig. 1). When more than 10% AEW was added to a microalgal culture, the microalgae exhibited immediate flocculation. The flocculation efficiency increased as a function of AEW concentration. The flocs that formed all settled within 10 min, regardless of the concentration of AEW, and maintained flocculation for 25 min after AEW addition. The maximum flocculation efficiency of 87.8% was observed with 20% AEW after 25 min of settling time. Papazi et al. [10] studied chemical harvesting of Chlorella minutissima and achieved a maximum flocculation efficiency of 80% after 3–4 h of incubation with sulfate (0.75 g/l) or chloride salts (0.5 g/l). These results indicated that AEW was more efficient than chemical flocculants, as it reduced the harvesting time. Previous studies on electrofloculation have reported that cations like Al³⁺ and Fe³⁺ introduced by electrolysis cause flocculation of microalgae [7]. Therefore, the chemical composition of AEW was analyzed. The result showed that AEW had high concentrations of Na⁺ (57.2 mg/l) and K⁺ (649 mg/l) (Table 1). The mineral ion concentration of AEW implies that the cation of electrolytic water is not generated via electrode oxidation, but that the mineral contained in the water is ionized. It is suggested that positively charged ionized minerals generated by water electrolysis induce flocculation of microalgae as a result of the opposite charge.

Factors Affecting the Flocculation Activity of AEW
Influence of cell concentration on flocculation efficiency. The relationship between the initial cell concentration and AEW dose on flocculation efficiency was investigated. Microalgae were harvested by centrifugation and resuspended in fresh medium to various biomass concentrations. Flocculation tests of these suspensions were carried out at
pH 8 over a range of AEW doses. As shown in Fig. 2A, a flocculation efficiency of 88.7% was achieved at a cell concentration of 2 g/l and 20% AEW after 20 min of settling time. Increasing the biomass concentration from 0.25 to 2 g/l at 20% AEW caused the flocculation efficiency to increase to 47.67%. Garzon-Sanabria et al. [25] observed that an 8-fold increase in biomass concentration required a 6-fold lower AlCl₃ dosage. Liu et al. [28] reported similar results in their study of freshwater microalgae harvested using flocculation induced by pH reduction. These results are consistent with previous findings that a high culture density in the medium aids flocculation, because cell-cell encounters are more frequent, leading to better aggregation [1]. Thus, flocculation activity increases with increasing cell concentrations.

**Effect of medium pH on flocculation activity.** The effect of pH on flocculation efficiency was investigated (Fig. 2B). The pH of the culture medium was varied from 6, which does not affect the survival of microalgae, to 9, which does not allow spontaneous flocculation. The initial pH of the microalgal culture medium was pH 8.4 ± 0.1 and it was adjusted to the appropriate pH with 0.5 N NaOH or 0.5 N HCl, and the flocculation test was carried out through addition of AEW at the indicated concentration. The highest flocculation efficiency was achieved at pH 9.0. At pH 7, at least twice the concentration of AEW was required to induce effective flocculation compared with the other pH conditions. These results demonstrate that the pH of the microalgal suspension has a significant effect on flocculation efficiency using AEW. Lee and Choi [26] investigated the effect of pH on the activity of Mg–sericite flocculant. They observed that the flocculation activity was stable at pH 9–11, but decreased at below pH 4. Ndikubwimana et al. [27] reported that the flocculation efficiency increased from 43.8% to 98.2% when the initial culture pH was reduced from 7.2 to 3. The significant influence of pH on flocculation activity occurs because a change of pH can have a significant effect on the physicochemical properties of algal cells and flocculant structures [28]. Changing the pH of the culture medium can affect the membrane surface charge of microalgal cells and alter the ionic forms of dissolved metals present in the culture medium through hydrolysis [29].

**Influence of ion concentration in the medium on flocculation activity.** To investigate whether flocculation by AEW is dependent on the ion concentration of the medium, flocculation of microalgae was tested in diluted and concentrated media with the same ionic composition. The microalgae were harvested by centrifugation and resuspended in F/2 medium at the indicated ion concentration, and a flocculation test was performed at pH 8. As shown in Fig. 2C, the optimal AEW dose for all ion concentrations was 20%, and the flocculation efficiency reached 80%. The concentration of ions in the medium appears to have little effect on flocculation efficiency at optimal AEW doses. This
result is inconsistent with previous reports that marine microalgae can exhibit altered activity of flocculants owing to the high ionic strength of seawater [12, 30].

Effect of AEW on Cell Integrity

Microalgal cell viability is an important factor in the harvesting process for biodiesel production. Some harvesting methods may lead to damaged cells and affect downstream processes for lipid extraction after harvesting [31]. To evaluate the effect of the flocculant (AEW) on cell membrane integrity, flocculated cells were stained with Evans blue and observed with light microscopy. With this method, living microalgal cells appear light green and yellow liquid within the cells can be seen clearly, whereas dead cells are dark green owing to the Evans blue dye and the yellow liquid within the cells is not visible. Flocculated cells were light green, as they were not stained by Evans blue and appeared similar to control cells. Fig. 3A shows flocculated cells that did not take up the Evans blue dye, indicating that their cell membranes remained intact.

The effects of AEW on the physiological activity of *Tetraselmis* sp. was estimated by comparing the growth rates of microalgae harvested by flocculation with those harvested by natural sedimentation. As shown in Fig. 3B, the yields of microalgae harvested by flocculation and natural sedimentation were similar. These results indicate that no cell lysis and no effect on the molecular function and structure of the photosynthetic apparatus had occurred during flocculation with AEW.

Recycling of the Flocculation Medium

It has been reported that medium recovered from flocculation could be reused [28, 32]. Reuse of flocculation medium can reduce the cost of biodiesel production by lowering the cost of microalgal cultivation and by saving water [33]. This flocculation method using AEW did not contaminate the growth medium, and could be reused after flocculation with pH neutralization and the addition of nutrients. Thus, the effect of medium reuse on microalgal growth was evaluated. As shown in Fig. 4, the growth curves of microalgal cells cultivated in the recycled medium were similar to those in fresh culture medium. These results indicate that the culture medium could be successfully recycled for microalgal culture. Normal growth of the microalga in the recycled medium indicates that the AEW components remaining after flocculation are not toxic to *Tetraselmis* sp.

Our results show that AEW is a potentially useful flocculant for harvesting the marine microalga *Tetraselmis* sp. AEW effectively flocculated the marine microalga because it contains positively charged ionized minerals (Na⁺ and K⁺). It is well known that the microalgal cell surface has a negative charge due to the presence of carboxylic and amine groups and they are electrostatically stabilized in suspension [34]. Therefore, positively charged ions or polymers may adsorb onto the microalgae owing to their opposite charge and cause flocculation by charge

**Fig. 3.** Cell integrity test after treatment with 20% alkaline electrolyzed water (AEW).

(A) Microscopic image of microalgae. (B) Effect on growth in natural sedimentation and flocculated microalgae cultivated in fresh medium.

**Fig. 4.** Growth curves of *Tetraselmis* sp. cultured in recycled and fresh media.
neutralization [35]. Hence, it can be hypothesized that positively charged ionized minerals of AEW bind to the negatively charged microalga through electrostatic attraction, and the surface charge is reduced or neutralized, resulting in the formation of flocs. We tested the factors that can affect microalgal flocculation. The culture density and pH had a significant effect on flocculation efficiency using AEW. On the other hand, the ion concentration had no significant effect on the efficiency of flocculation with AEW. The maximum flocculation activity of 88.7% was observed at 20% AEW, pH 8, and 2 g/l of biomass. Morphological observations under a microscope showed that the microalgal cells were intact, without signs of lysis, after flocculation. Furthermore, medium recovered from flocculation could be recycled, reducing the demand for water and the cost of biodiesel production from microalgae. Thus, this flocculation technique with AEW is easy to use and cost-effective for application in industrial-scale microalgal biodiesel production. However, further study is needed to elucidate the mechanism of flocculation.

**Acknowledgments**

This research was supported by collective research grants from the Korea Institute of Ocean Science & Technology (PE99511). Moreover, the study in this paper was supported by the “Marine Biotechnology Program” funded by the Ministry of Ocean and Fisheries (PM60230 and PM60110), Korea.

**Conflict of Interest**

The authors have no financial conflicts of interest to declare.

**References**


