Membrane Water Treatment, *Vol. 9, No. 3 (2018) 147-153*
DOI: https://doi.org/10.12989/mwt.2018.9.3.147

Flocculation of microalgae using extracellular polymeric substances (EPS) extracted from activated sludge

Dandan Dong[1], Dongmin Seo[2], Sungkyu Seo[2] and Jae Woo Lee*[1]

[1]*Program in Environmental Technology and Policy, Korea University, Sejong, 30019, Republic of Korea*
[2]*Department of Electronics and Information Engineering, Korea University, Sejong, 30019, Republic of Korea*

(Received December 2, 2017, Revised May 1, 2018, Accepted May 2, 2018)

Abstract. This study investigates the role of microbial extracellular polymeric substances (EPSs) as bioflocculants to harvest microalgae (water-microalgae separation). The EPS extracted from waste activated sludge (WAS) by heat extraction were fractionated into soluble EPS (S-EPS), loosely-bound EPS (LB-EPS) and tightly-bound EPS (TB-EPS) forms. All the EPSs facilitated the flocculation of microalgal cells from stable growth medium. Of those EPSs, the TB-EPS showed the highest flocculating activity (FA) resulting in the substantial decrease in the amount of EPS added in terms of total organic carbon (TOC) during flocculation. The FA of microalgae was improved with the increase in TB-EPS dose, however, excessive dose of TB-EPS adversely affected it due to destabilization. Both LB- and TB-EPS could be utilized for flocculating microalgae as a sustainable option to the existing chemical-based flocculants. In addition to the conventional assessments, the effectiveness of the two bioflocculants for floc forming was also confirmed using a novel assessment of lens-free shadow imaging technique (LSIT), which was firstly applied for the rapid and quantitative assessment of microalgal flocculation.

Keywords: microalgae; extracellular polymeric substances (EPS); bioflocculant; activated sludge; lens-free shadow imaging technique (LSIT)

1. Introduction

Currently, microalgae are the focus of attention as a source of renewable biomass. It can be used as a source of biofuel or food for human and farm animals due to its highly valuable carbon content, i.e., lipids and carbohydrates and higher rate of biomass reproduction compared with traditional food crops (Lee 2011, Skrede 2011, Ibrahim *et al*. 2015). In addition to intensive cultivation, it has been used for wastewater treatment, particularly, for nutrient removal based on microalgal assimilation (Feng *et al*. 2011). In spite of these benefits, the relatively high overall cost indicates that commercial production of microalgae is only economically feasible for a few high value market products such as natural pigments, food supplements, or poly-unsaturated fatty acids. For the other products with a relatively low market value such as animal feed or biofuel, the production cost should be decreased by at least one order of magnitude (Sing *et al*. 2013).

Harvesting, i.e., separation of water and microalgae, contributes to a large portion of the total cost. A relatively large supply of energy and chemicals is needed to separate the microalgae from stable suspension because of the relatively small size, negative charge and in some cases mobility of algae during separation (Tenny *et al*. 1968). Harvesting process for the algal culture crucially affects the energy consumption and cost approximately accounting for 20-30% of the total energy required and cost (Lardon *et al*. 2009, Grima *et al*. 2003). Thus, the harvesting process should be developed in a more sustainable way by lowering the energy consumption and excluding harmful chemicals in the valuable products (Chen *et al*. 2011).

Flocculation is a prerequisite or desirable for enhancing the performance of most harvesting technologies, such as centrifugation, gravity settling, flotation and filtration. Flocculation uses various types of flocculants such as inorganic (typically cation-based) chemicals, organic polymers and naturally occurring bioflocculants (Shih *et al*. 2001). Flocculation using either inorganic or organic chemicals is considered easy and effective as it handles a large volume of culture without excessive energy inputs (Lee *et al*. 2011). However, it is not sustainable or cheap to harvest microalgae due to the extra operational costs required for removal of flocculant residues (Schenk *et al*. 2008).

In recent years, flocculation using bioflocculants as a means to separate the microalgae from the liquid medium has gained considerable attention in research. The bioflocculants are the biologically derived flocculants from natural sources including bacteria, algae and fungi (Ugbenyen 2014, He *et al*. 2011). In recent years, extracellular polymeric substances (EPS) have been shown to play a similar role as bioflocculant in activated sludge (AS) by binding with a variety of microorganisms (Sun *et al*. 2015). EPS are composed of complex organic substances including carbohydrates and proteins as major constituents and humic substances, uronic acids and nucleic acids as minor quantities (Karapanagiotis 1989, Frølund *et al*. 1996).

*Corresponding author, Professor
E-mail: jaewoo@korea.ac.kr

ISSN: 2005-8624 (Print), 2092-7037 (Online)

These components of EPS are surface-active and aggregate algal cells by binding together. The EPS in AS flocs can be differentiated into bound and soluble EPS. Soluble EPS are weakly bound to cells or dissolved in solution, while the bound EPS present as a capsule surrounding the bacterial cell wall within the sludge floc. The bound EPS are further differentiated tightly bound EPS (TB-EPS), which are stably bound onto the cell surface and loosely bound EPS (LB-EPS) that is contained in a dispersible slime layer (Sheng 2010, Zhang *et al*. 2014).

In order to qualitatively and quantitatively analyze the impact of the EPS on flocculation of microalgae at real time, lens-free shadow imaging technique (LSIT) was applied in this study. In most cases, microscope linked with charge coupled devices (CCDs) has been used for observation or enumeration of micro-cell or flocs (Hiraoka *et al*. 1987). This method requires a specific optical platform including microscope equipped with optical lens for enhancing the resolution of the micro-scale images. Compared to this method, LSIT is considered to be cost-effective, compact, less labor-intensive and less time consuming since it does not require the specific high quality optical lens. Instead, it needs a simple platform consisting of LED light source and complementary metal oxide semiconductor (CMOS) detectors for identifying shadow images of individual microalgal cell or flocs. LSIT provides larger-scale spatial information than microscope, numbers, size and the state of a cell or flocs by acquiring the inherent their diffraction pattern without the use of any lenses or other bulky optical components (Kim *et al*. 2012). Recently, this technique has been applied to count and analyze the micro-sized samples in biotechnology such as estimation of number and activity of blood cell, animal cell and bacteria (Seo *et al*. 2010, Seo *et al*. 2018, Lee *et al*. 2014). However, to our understanding, its application has been little attempted to environmental engineering to date.

The goal of this study was to investigate the role of various EPS fractions extracted from waste activated sludge (WAS) as a bioflocculant to flocculate microalgae (*Scenedesmus sp*) from a growth medium. Specific goals include examination of the content of LB-EPS and TB-EPS in WAS, to evaluate the effectiveness of each EPS in floc formation of microalgae and to determine the optimal dosage of the EPS with the highest flocculation capability among the EPSs. Furthermore, the utility of lens-free shadow imaging technique was investigated as a real-time quantitative method for estimating the EPS capacity for flocculation of microalgae.

2. Materials and method

2.1 Preparation of microalgae and bioflocculant

2.1.1 Cultivation of microalgae

Scenedesmus sp. has a rapid growth rate and contains a larger amount of oleic acid compared with the other microalgal species (Prabakaran 2012, Papazi *et al*. 2010). The microalgae were cultured in Erlenmeyer flasks filled with 250 mL BG-11 culture medium that was prepared in the laboratory (Waterbury and Stanier 1981). The culture flasks were continuously stirred at 20 rpm by a rotary shaker placed in an incubator (HANBAEK HB201SF, Korea). The incubator was equipped with fluorescent light (illumination intensity of 60 μmol m-2 s-1) and its temperature was maintained at 20 ± 1°C. Growth state of the microalgae was monitored by measuring the optical density (OD) at 680 nm. The algal culture was incubated until the OD of microalgae reached 0.5. Thereafter, microalgal cultures were removed for experiments after examining their viability microscopically.

2.1.2 Cultivation of activated sludge

Activated sludge (AS) was derived from a wastewater treatment plant (WWTP) using the sequencing batch reactor (SBR) process in Chungbuk province in Korea. The activated sludge samples were directly taken from the SBR process during aeration. We transferred the AS sample to a lab-scale SBR (6L). The AS was cultivated under room temperature in the SBR by replacing 2L of the growth medium every day. The composition of culture medium was prepared with the following composition: Glucose, 500 mg/L; Peptone, 400 mg/L; NH3Cl, 200 mg/L; K2HPO4, 45 mg/L; CaCl2·2H2O, 30 mg/L; MgSO4·7H2O, 50 mg/L; FeSO4·7H2O, 20 mg/L; and 1 mL of trace mineral solution. The composition of trace metal solution in culture medium were in the following concentrations: H3BO3, 50 mg/L; MnSO4·H2O, 50 mg/L; (NH4)6Mo7O2·H2O, 50 mg/L; ZnCl2, 50 mg/L; CuCl2, 50 mg/L; AlCl3, 50 mg/L; NiCl2, 50 mg/L; CoCl2·6H2O, 0.05 mg/L. The AS was cultivated under room temperature in the SBR by replacing 2L of the growth medium every day. Solid retention time (SRT) and hydraulic retention time (HRT) were set at 10 days and 12 h, respectively. The concentration of mixed liquor suspended solids (MLSS) during aeration was maintained at around 3,000 mg/L. The MLSS obtained from the reactor for SRT control was prepared for EPS extraction.

2.1.3 EPS extraction from activated sludge

The physical extraction methods include ultrasonication, centrifugation, use of cation-exchange resins and heating, whereas the chemical methods involved treatment with ethylenediamine tetraacetic acid (EDTA) and HCHO/NaOH mixture (Liu and Fang 2002a). Researchers have optimized the extraction methods. However, no standardized method is currently available. In this study, EPS in the AS cultivated in a lab-scale SBR were extracted by a heat extraction method (Laspidou *et al.* 2002). Three different types of EPS including soluble EPS (S-EPS), loosely-bound EPS (LB-EPS) and tightly-bound EPS (TB-EPS) were prepared. The 400 mL AS sample taken from the SBR was centrifuged at 4,000g for 15 min and the supernatant was recovered as S-EPS. The sludge pellet was resuspended by adding the 400 mL growth medium for AS, which was pre-heated rapidly to ensure a warm temperature for the sludge suspension. The mixture was heated at 50˚C for 1 min in a heating bath. It was centrifuged again at 4,000g for 15 min and the supernatant was recovered as the LB-EPS of the sludge. The sludge pellet leftover in the centrifuge tube was resuspended and heated at 60˚C for 30 min in the heating bath. After centrifugation, the supernatant was recovered as the TB-EPS of the AS.

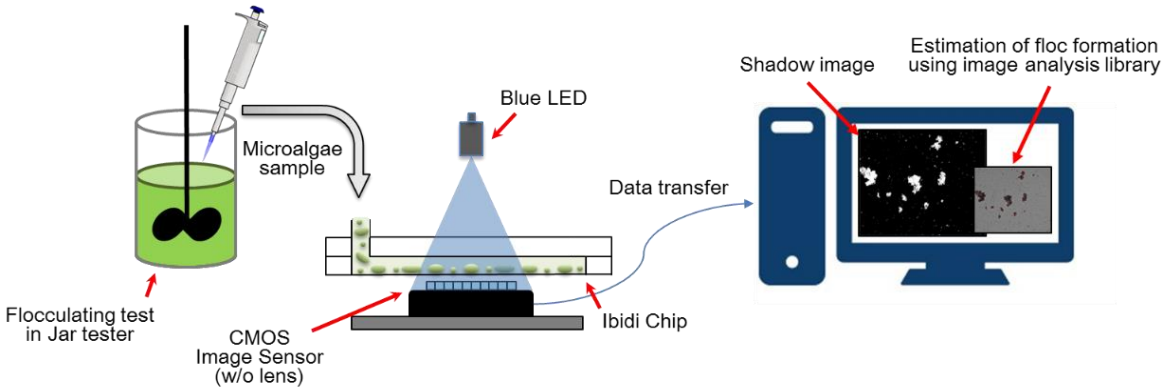

Fig 1. Experimental setup for floc forming analysis using LSIT

2.2 Jar test

In order to examine the effectiveness of EPS as bioflocculants, flocculation tests were carried out in a Jar tester. Three different EPS fractions of S-EPS, LB-EPS and TB-EPS were prepared, respectively. The 160 mL algal culture and 40 mL of each EPS were added into a 500 mL beaker placed in a Jar tester. In the flocculation experiment, the mixed samples were stirred at 100 rpm for 2 min, followed by slower mixing at 20 rpm for 20 min at room temperature. The flocculated algal cultures were allowed to settle and the aliquot at a height of two-thirds from the bottom was obtained to measure OD. A control experiment was conducted under the same conditions without adding bioflocculant. The effectiveness of EPS as a bioflocculant was evaluated in terms of flocculating activity (FA) based on the following equation.

$$FA\ (\%) = (A\text{-}B)/A \times 100 \quad (1)$$

where, A and B is the OD of control and treated sample, respectively.

Once the best EPS fraction was selected based on FA, its optimal dosage was determined by conducting the same flocculation and settling tests using different amounts of the EPS: 30 ml, 40 ml, 60 ml and 80 ml.

2.3 Analytical methods

The optical density (OD) was measured at 680 nm using a spectrophotometer (HACH DR/2010). In order to estimate the EPS content, the total organic carbon (TOC) of each EPS fraction was measured using a TOC analyzer (5000A, SHIMADZU) after filtration with 0.45 μm PTFE membrane filter. The TOC and conductivity were also measured for the supernatant samples to estimate the EPS utilized for flocculation. The zeta potential of the control (microalgae only) and microalgal mixture with the EPS was measured during the flocculation experiment using a zeta potential analyzer (Malvern Zetasizer, Malvern, UK) in order to investigate changes in the surface charge of floc due to EPS addition. All the measurements for TOC and zeta potential were conducted in duplicate. The total suspended solids (TSSs) and volatile suspended solids (VSSs) were analyzed during cultivation of activated sludge according to the standard methods (APHA *et al.* 1998).

2.4 Lens-free shadow imaging technique (LSIT)

Floc formation of microalgae was evaluated using LSIT. The mixture of microalgae and EPSs was continuously stirred at 20 rpm for 32 min which was slightly longer than the flocculation time conducted during the flocculation experiment mentioned above. The samples (100 μL) were drawn from the beaker placed in the Jar test at different times in a glass tube. The samples were placed plastic chip (Ibidi, Germany) where the microalgal floc was monitored by a lens-free shadow image analytical unit, which was composed of a light source and image sensor (Fig. 1). The LED light source was placed on the upper side of the plastic chip and the CMOS image sensor (EO-5012, Edmund optics, U.S.A.) on the bottom of the plastic chip. This unit transmits the shadow images obtained using the CMOS image sensor to a computer for analysis of floc size and number based on a self-developed automatic floc analysis program using the image analysis library in MATLAB (MathWorks®, U.S.A.).

3. Results and discussion

3.1 Characteristics of microalgae and EPS

The optical density (OD) of microalgae (*Scenedesmus sp.*) in this study increased up to 1.0 during cultivation. As a preliminary test, the settling behavior of microalgae with different ODs ranging from 0.5 to 1.0 was investigated for 5 h. The flocculated algal cultures were allowed to settle and the aliquot was taken at a height of two-thirds from the bottom, then OD was measured every an hour. The settling behavior of microalgae was getting worse as the initial OD decreased and the OD 0.5 showed the worst settling behavior (data not shown). Hence, in order to investigate the effectiveness of EPS as a bioflocculant more accurately, the initial OD value of microalgae was set at 0.5 in this study. The EPS extracted from the AS was fractionated into S-EPS, LB-EPS and TB-EPS. Individual EPS content was expressed in terms of TOC concentration (McSwain *et al.* 2005) and is shown in Table 1. The content of the TB-EPS was significantly higher than the other EPS. S-EPS showed the least content among all the fractionated EPS and was nearly 40 times lower than that of the TB-EPS.

Table 1 TOC and conductivity of the different EPS fractions (mean ± standard deviation)

Sample	TOC (mg/g VSS)	Conductivity (μs/cm)
S-EPS	2.87 ± 0.19	0.84 ± 0.42
LB-EPS	6.40 ± 0.63	0.84 ± 0.95
TB-EPS	107.78 ± 2.48	1.07 ± 0.53

A higher content of the TB-EPS suggested that the strongly bound EPS in AS was dominant and extracted under temperatures greater than 60˚C. In spite of relatively large variation in data (with higher standard deviation), the conductivity of the TB-EPS appeared to be higher than others indirectly indicating the potential presence of additional cations available for charge neutralization during flocculation (Table 1) (Yu *et al.* 2009).

3.2 Effects of EPS types on flocculation and settling of microalgae

3.2.1 Flocculating activity depending on EPS fractions

Flocculation and settleability of microalgae were enhanced by the addition of EPS fractions compared with the control (without the bioflocculant). As the flocculation time increased, the OD of the supernatant in each sample decreased and therefore, the flocculating activity (FA) increased. Fig. 2 shows the FA of the various EPS added to the microalgal culture. The addition of TB-EPS resulted in the highest FA (Yu *et al.* 2009). The FA of the LB-EPS increased rapidly up to 62% during an earlier settling time (< 2 h). On the other hand, the TB-EPS increased continuously and surpassed that of the LB-EPS after 2 h. Both the LB- and TB-EPS exhibited a fairly satisfactory performance with a higher final FA accounting for 85% and 91%, respectively.

The role of EPS in bioflocculation is explained by two mechanisms of bridging and charge neutralization. Bridging mechanism is associated with organic type and content, however charge neutralization is related to the concentration and type of cations (Yu *et al.* 2009). In case of EPS, the bridging mechanism is predominantly found for flocculation. As the EPS, mostly consisting of organic substances, is attached to the algal cell, it binds algal cells together and shortens the distance between cells in the stable suspension acting like a glue (Sheng *et al.* 2010). The TB-EPS containing a greater amount of TOC content accelerates floc formation and consequently enhances FA. Despite a lower TOC, the LB-EPS exhibited similar FA probably due to the presence of better types of organics for binding with the microalgal cells. A greater decrease (from 310 to 12 mg/L) in TOC concentration of the TB-EPS sample suspension represented higher EPS utilization in microalgal floc formation. In contrast to the TB-EPS, the TOC concentration of the LB- and SB-EPS decreased from 19 and 8 mg/L to 6 and 3 mg/L, respectively.

3.2.2 Zeta potential of microalgal floc

Zeta potential indicates the electrical charge of

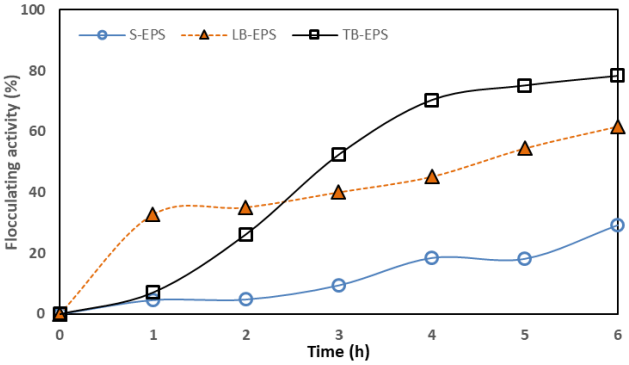

Fig. 2 Flocculating activity of microalgae (Scenedesmus sp.) depending on EPS type

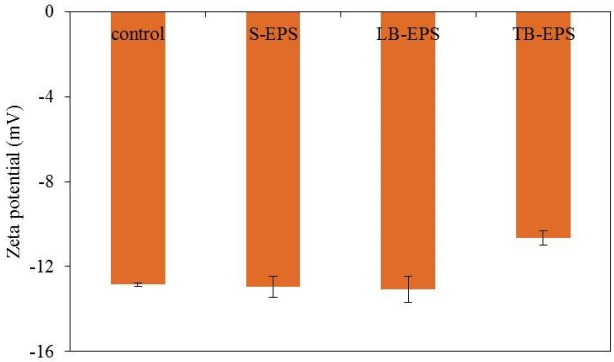

Fig. 3 Zeta potential of microalgae in suspension after flocculation

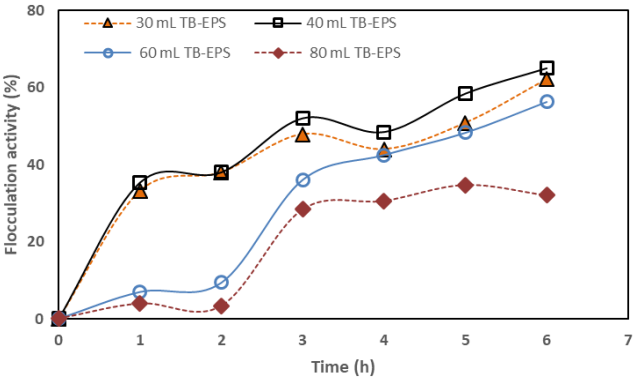

Fig. 4 FA of microalgae with different dosage of TB-EPS

microalgal cell surface in a suspension. A high negative or positive zeta potential leads to strong electrical repulsion between the microalgae resulting in a stable suspension. Conversely, a lower zeta potential leads to aggregation of microalgae. Fig. 3 shows the zeta potential of Scenedesmus sp. culture after flocculation with the addition of different EPS fractions. The zeta potential remained negative in the range of -10.65 to -13.06 mV for all samples and the value for the TB-EPS sample was slightly higher than the others (Fig. 3). Higher (less negative) zeta potential of the TB-EPS sample supported a higher FA, as shown in Fig. 1 (Gong *et al.* 2009). Increase in zeta potential by the addition of TB-EPS was closely associated with the charge neutralization, which was supported by the higher conductivity of TB-EPS compared with the other EPS (Table 1). Higher conductivity

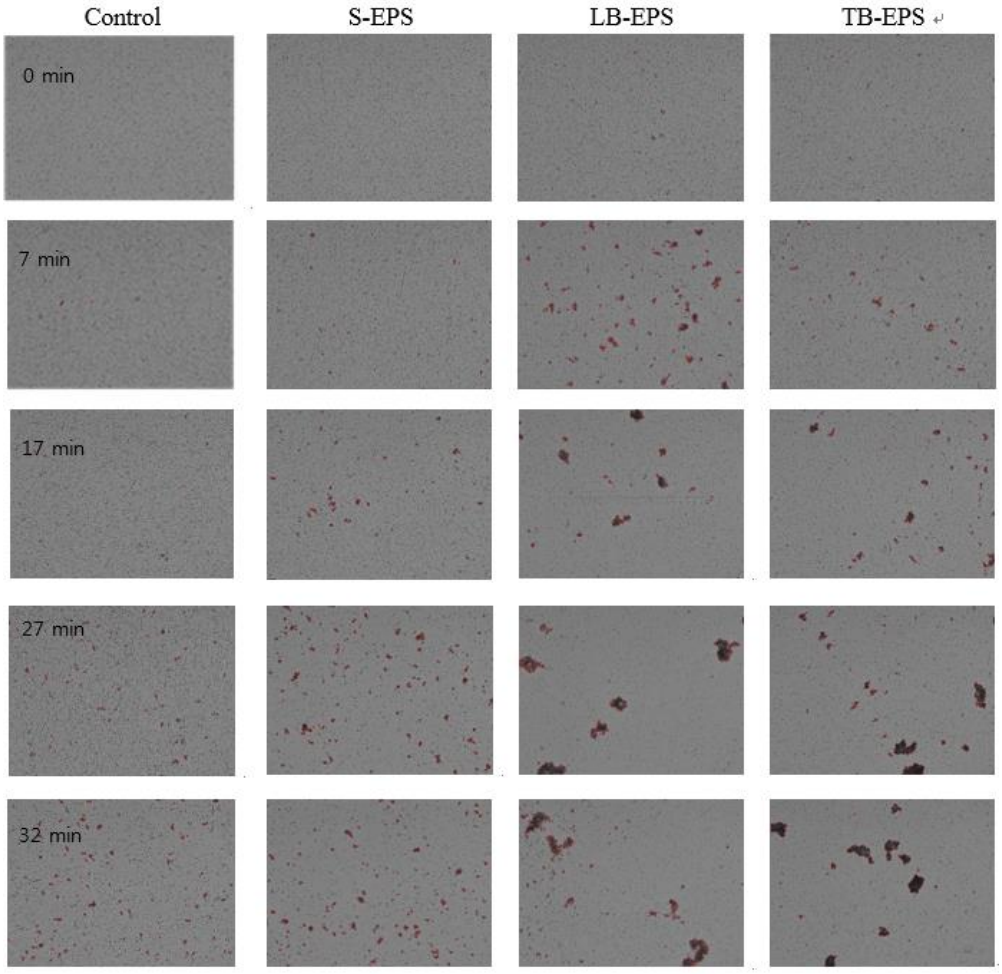

Fig. 5 Shadow image of microalgae culture and their flocs formed for 32 min due to addition of EPSs. From left to right, control, S-EPS, LB-EPS and TB-EPS

of the TB-EPS suggested potential generation of additional cations leading to charge neutralization. Nevertheless, the charge neutralization by TB-EPS played a minor role in microalgal flocculation using EPS. The TB-EPS showed superior FA compared with the other EPS, however, the zeta potential was still negative indicating that the electrostatic repulsion between microalgae was not significantly diminished by the addition of TB-EPS.

3.2.3 Optimal dosage of TB-EPS for flocculation

Since the TB-EPS was the most effective bioflocculant among the EPS fractions, its optimal dosage was determined by adding different amounts ranging from 30 mL to 80 mL. The effect of different dosages of TB-EPS on FA is shown in Fig. 4. Addition of 30 mL and 40 mL of TB-EPS resulted in a significantly higher FA until 3 h of settling time. Thereafter, the addition of 60 mL TB-EPS yielded a similar FA, however, a higher dosage (80 mL) had no additional effect on FA. Addition of the TB-EPS lower than 60 mL yielded a higher FA than 85% during the settling experiment, however, the FA due to 80 mL dosage was limited at 72%. The adverse effect of excessive levels of EPS were attributed to destabilization of microalgal flocs (Guo *et al*. 2015).

3.3 Assessment of flocculation using lens-free shadow imaging technique (LSIT)

We propose a novel method of LSIT to visualize the microalgal floc formation and to quantitatively analyze the effective floc area and the number of flocs. Fig. 5 illustrates the shadow images of microalgal flocs over time and the series of figures show changes in the microalgal culture or flocs in control, S-EPS, LB-EPS and the TB-EPS samples, respectively. As shown in Fig. 5, the individual spots in the shadow image indicated individual microalgal cultures.

Two or more overlapped spots in the shadow image are detected and marked with a red boundary, which was defined as an effective floc in this study. As the flocculation time increased, the size of effective flocs markedly increased in the LB-EPS and TB-EPS samples compared with control. At the end of flocculation, the number of effective flocs in control was higher than in the LB- and TB-EPS. However, the total area of the effective flocs in the LB- and TB-EPS samples was substantially bigger than in the control demonstrating that LB- and TB-EPS enhanced the flocculation leading to microalgal floc agglomerates. The area and number of the effective flocs were automatically calculated by counting their number and measuring the area of internal pixels in the red boundaries as shown in Figs. 6 and 7, respectively.

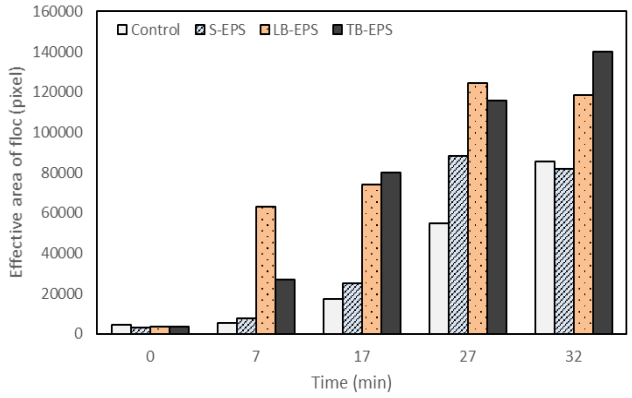

Fig. 6 Effective floc area of microalgae formed during flocculation depending on EPS types

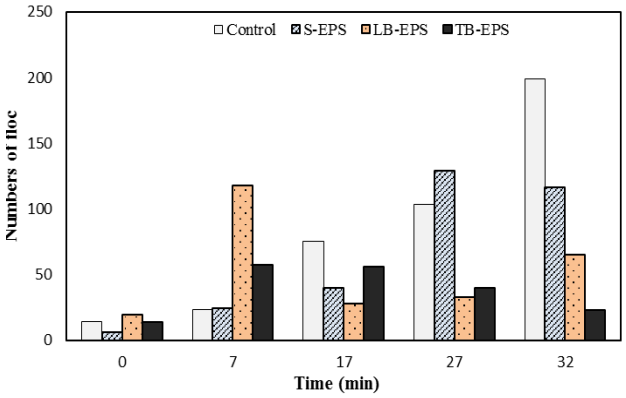

Fig. 7 Number of flocs of microalgae formed during flocculation depending on EPS types

Increase in the effective floc area was significant in the LB-EPS sample at earlier flocculation times (< 10 min). However, it was more significant in the TB-EPS, which attained a similar area at a later time (Fig. 6). The effective floc area of the TB-EPS sample accounted for 139,954 pixels at the end of flocculation (32 min), which was 4 times greater than that of the control (36,813 pixels). The effective area of floc with LB-EPS addition was similar to that of the TB-EPS, however, the S-EPS was similar to that of the control. The maximum floc size observed in the LB- and TB-EPS was 40,509 and 56,769 pixels, respectively. Both are at least 10 times greater than those of the S-EPS and control. The number of effective flocs increased abruptly in the LB-EPS sample in 2 min, followed by a decrease until the end of flocculation suggesting that small flocs formed earlier and agglomerated during the remainder of the flocculation time (Fig. 7). Floc formation in the TB-EPS was slightly slower than in LB-EPS despite a larger floc size at the end of flocculation. The number of flocs in the control and in the S-EPS increased until the end of flocculation requiring a longer flocculation time for increased floc size. The overall results indicated that both LB-EPS and TB-EPS were utilizable as a bioflocculant. A single-step thermal extraction under higher temperature (> 60°C), which is a condition for TB-EPS, was recommended to recover additional effective EPSs. Further, the LSIT first attempted in this study provided valuable information about microalgal floc formation. LSIT facilitates rapid and quantitative assessment of methods for flocculation.

4. Conclusions

The role of the EPS thermally extracted from activated sludge was investigated as a bioflocculant of microalgal suspension. Similarly, the superior potential of LB- and TB-EPS were confirmed by several analyses such as flocculation-settling experiment, zeta potential and lens free shadow imaging technique (LSIT). Both LB-EPS and TB-EPS showed a relatively satisfactory performance with higher flocculating activity. The TB-EPS containing an abundance of EPS exhibited the highest flocculating activity, however, at excessive doses, it destabilized flocculation. The LB-EPS could be also utilized as a bioflocculant for flocculating microalgae despite smaller EPS content in terms of TOC indicating that specific composition as well as abundance of the EPS might affect FA. Thus, single step thermal method was recommendable to recover both EPSs together. The LSIT first used for microalgal flocculation in this study demonstrated the effectiveness of LB- and TB-EPS for enhancing flocculation of microalgae at real time. The thermal extraction of EPS from activated sludge is a sustainable and cost-effective approach to microalgal harvesting. Furthermore, the LSIT represents a quick and quantitative method for the estimating of particle and microalgal flocculation.

Acknowledgments

This subject was supported by the Korea Agency for Infrastructure Technology Advancement (KAIA) grant funded by the Ministry of Land, Infrastructure and Transport (Grant 17CTAPC128926-01) and "Waste to Energy and Recycling Human Resource Development Project" by Ministry of Environment.

References

Chen, C., Yeh, K., Aisyah, R., Lee, D. and Chang, J. (2011), "Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review", *Bioresource Technol.*, **102**(1), 71-81.

Feng, Y., Li, C. and Zhang, D. (2011), "Lipid production of Chlorella vulagaris cultured in artificial wastewater medium", *Bioresource Technol.*, **102**(1), 101-105.

Frølund, B., Palmgren, R., Keiding, K. and Nielsen, P.H. (1996), "Extraction of extracellular polymers from activated sludge using a cation exchange resin", *Water Res.*, **30**(8), 1749-1758.

Gong, J.L., Wang, B., Zeng, G.M., Yang, C.P., Niu, C.G., Niu, Q. Y. and Liang, Y. (2009), "Removal of cationic dyes from aqueous solution using magnetic multi-wall carbon nanotube nanocomposite as adsorbent", *J. Hazard. Mater.*, **164**(2), 1517-1522.

Grima, E.M., Belarbi, E.H., Fernández, F.A., Medina, A.R. and Chisti, Y. (2003), "Recovery of microalgal biomass and metabolites: Process options and economics", *Biotech. Adv.*, **20**(7-8), 491-515.

Guo, J., Yu, J., Xin, X., Zhou, C., Cheng, Q., Yang, H. and Nengzi

L. (2015), "Characterization and flocculation mechanism of a bioflocculant from hydrolyzate of rice stover", *Bioresource Technol*., **177**, 393-397.
He, B., Ye, J., Yin, H., Qin, H., Yu, L., Zhang, N. and Peng, H. (2011), "Production and characteristics of bioflocculant from azotobacter", *Proceedings of the 5th International Conference on Bioinformatics and Biomedical Engineering (iCBBE 2011),* Wuhan, China, May.
Hiraoka, Y., Sedat, J.W. and Agard, D.A. (1987), "The use of a charge-coupled device for quantitative optical microscopy of biological structures", *Science*, **238**(4823), 36-41.
Ibrahim, R.I., Mohammad, A.W. and Wong, Z.H. (2015), "Optimization of POME treatment process using microalgae and ultrafiltration", *Membr. Water Treat*., **6**(4), 293-308.
Karapanagiotis, N.K., Rudd, T., Sterritt, R.M. and Lester, J.N. (1989), "Extraction and characterisation of extracellular polymers in digested sewage sludge", *J. Chem. Technol. Biotech*., **44**(2), 107-120.
Kim, S.B., Bae, H., Koo, K.I., Dokmeci, M.R., Ozcan, A. and Khademhosseini, A. (2012), "Lens-free imaging for biological applications", *J. Lab. Automation*, **17**(1), 43-49.
Lardon, L., Helias, A., Sialve, B., Steyer, J.P. and Bernard, O. (2009), "Life-cycle assessment of biodiesel production from microalgae", *Environ. Sci. Technol*., **43**(17), 6475-6481.
Laspidou, C.S. and Rittmann, B.E. (2002), "A unified theory for extracellular polymeric substances, soluble microbial products and active and inert biomass", *Water Res*., **36**(11), 2711-2720.
Lee, S., Kim, S., Kim, J., Kwon, G., Yoon, B. and Oh, H. (1998), "Effects of harvesting method and growth stage on the flocculation of the green alga Botyrococcus braunii", *Lett. Appl. Microbiol*. **27**(1), 14-28.
Lee, D.H. (2011), "Algal biodiesel economy and competition among bio-fuels", *Bioresource Technol*., **102**(1), 43-49.
Lee, J., Kwak, Y.H., Paek, S.H., Han, S. and Seo, S. (2014), "CMOS image sensor-based ELISA detector using lens-free shadow imaging platform", *Sensor. Actuat. B: Chem*., **196**(1), 511-517.
Liu, H. and Fang, H.H. (2002), "Extraction of extracellular polymeric substances (EPS) of sludge", *J. Biotechnol*., **95**(3), 249-256.
McGarry, M.G. (1970), "Algal flocculation with aluminum sulfate and polyelectrolytes", *Journal (Water Pollution Control Federation)*, **42**(5), R191-R201.
McSwain, B.S., Irvine, R.L., Hausner, M. and Wilderer, P.A. (2005), "Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge", *Appl. Environ. Microbiol*., **71**(2), 1051-1057.
Papazi A., Makridis P. and Divanach P. (2010), "Harvesting Chlorella minutissima using cell coagulants", *J. Appl. Phycol*., **22**(3), 349-355.
Prabakaran, P. and Ravindran, A.D. (2012), "Scenedesmus as a potential source of biodiesel among selected microalgae", *Current Sci*., 616-620.
Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J. H., Posten, C. and Hankamer, B. (2008), "Second generation biofuels: High-efficiency microalgae for biodiesel production", *Bioenergy Res*., **1**(1), 20-43.
Seo, D., Oh, S., Lee, M., Hwang, Y. and Seo, S. (2017), "A field-portable cell analyzer without a microscope and reagents", *Sensors*, **18**(1), 85.
Seo, S., Isikman, S.O., Sencan, I., Mudanyali, O., Su, T.W., Bishara, W. and Ozcan, A. (2010), "High-throughput lens-free blood analysis on a chip", *Analytical Chem*., **82**(11), 4621-4627.
Sheng, G.P. and Li, X.Y. (2010), "Extracellular polymeric substances (EPS) microbial aggregates in biological wastewater treatment systems: A review", *Biotechnol. Adv.*, **28**(6), 882-894.
Shih, I., Van, Y., Yeh, L., Lin, H. and Chang, Y. (2001), "Production of a biopolymer flocculant from Bacillus licheniformis and its flocculation properties", *Bioresour. Technol*., **78**(3), 267-272.
Sing, S. F., Isdepsky, A., Borowitzka, M. A. and Moheimani, N. R. (2013), "Production of biofuels from microalgae", *Mitigation Adaptation Strategies Global Change*, **18**(1), 47-72.
Skrede, A., Mydland, L.T., Ahlstrom, O., Reitan, K.O., Gislerod, H.T. and Overland, M. (2011), "Evaluation of microalgae as sources of digestible nutrients for monogastric animals", *J. Anim. Sci*., **20**(1), 131-142.
Sun, P., Hui, C., Yang, S., Wan, L., Zhang, Q. and Zhao, Y. H. (2015), "Revealing the characteristics of a novel bioflocculant and its flocculation performance in Microcystics aeruginosa removal", *Scientific Reports*, **5**, 17465.
Tenny M.W., Echelberger W.F. Jr., Schuessler R.G. and Pavoni J.L. (1969), "Algae flocculation with synthetic organic polyelectrolytes", *Appl. Microbiol*., **18**(6), 965-971.
Ugbenyen, A.M. and Okoh, A.I. (2014), "Characteristics of a bioflocculant produced by a consortium of Cobetia and Bacillus species and its application in the treatment of wastewaters", *Water SA*, **40**(1), 139144.
Waterbury, J.B. and Stanier, R.Y. (1981), "Isolation and growth of cyanobacteria from marine and hypersaline environments", *The Prokaryotes*, Springer, Germany, 221-223.
Yu, G.H., He, P.J. and Shao, L.M. (2009), "Characteristics of extracellular polymeric substances (EPS) fractions from excess sludges and their effects on bioflocculability", *Bioresource Technol*., **100**(13), 3193-3198.
Zhang, A., Liu, Z., Chen, Y., Kuschk, P. and Liu, Y. (2014), "Effects of EPS on membrane fouling in a hybrid membrane bioreactor for municipal wasterwater treatment", *Membr. Water Treat*., **5**(1), 1-14.

CC