Influence of extracellular polysaccharides (EPS) produced by two different green unicellular algae on membrane filtration in an algae-based biofuel production process

Takaki Matsumoto, Hiroshi Yamamura, Jyunpei Hayakawa, Yoshimasa Watanabe and Shigeaki Harayama

ABSTRACT

In the present study, two strains of green algae named S1 and S2, categorized as the same species of *Pseudo-coccomyxa ellipsoidea* but showing 99% homology, were cultivated under the same conditions and filtrated with a microfiltration membrane. On the basis of the results of the extracellular polysaccharides (EPS) characteristics of these two green algae and the degree of fouling, the influence of these characteristics on the performance of membrane filtration was investigated. There was no difference in the specific growth rate between the S1 and S2 strains; however, large differences were seen in the amount and quality of EPS between S1 and S2. When the S1 and S2 strains were filtered with a membrane, the trend in the increase in transmembrane pressure (TMP) was quite different. The filtration of the S1 strain showed a rapid increase in TMP, whereas the TMP of the filtration of the S2 strain did not increase at all during the operation. This clearly demonstrated that the characteristics of each strain affect the development of membrane fouling. On the basis of the detailed characterization of solved-EPS (s-EPS) and bound-EPS (b-EPS), it was clarified that s-EPS mainly contributed to irreversible fouling for both operations and the biopolymer-like organic matter contained in b-EPS mainly contributed to reversible fouling.

Key words | algal biofuel, biopolymer, extracellular polysaccharides (EPS), membrane filtration, membrane fouling

Takaki Matsumoto
Hiroshi Yamamura (corresponding author)
Jyunpei Hayakawa
Shigeaki Harayama
Department of Science and Engineering,
Chuo University,
1-13-27 Kasuga,
Bunkyo-ku,
Tokyo,
Japan
E-mail: *yamamura.10x@g.chuo-u.ac.jp*

Yoshimasa Watanabe
Research and Development Initiative,
Chuo University,
1-13-27 Kasuga,
Bunkyo-ku,
Tokyo,
Japan

INTRODUCTION

Biofuel has been attracting attention owing to issues such as global warming and the soaring price of fossil resources. In particular, algae biofuel has created considerable interest in recent years as a renewable energy resource for the next generation because green algae have several benefits. They have 10 times higher photosynthetic capacity than plants (Schenk *et al.* 2008). Furthermore, they are capable of growing in the ocean as well as on land, and pose no competition with food production.

Algae biofuel is produced through four processes: cultivation, harvest, cell disruption, and oil refinement. The costs of harvesting microalgae biomass can be a major component of production, accounting for up to 20–30% of the total cost (Molina Grima *et al.* 2003). Carbon dioxide emissions from the separation of algal cells from the culture solution accounts for more than half of all the CO2 emissions from the production process and is a serious barrier to this technology becoming a practical energy resource. There are a number of processes available for the separation of algal cells from the culture solution, each having drawbacks. If sedimentation is used, plenty of time is needed for the complete separation owing to both the small size and low density of the cells (Henderson *et al.* 2007). The cost of centrifugation is reported to be extremely high, and it is difficult to achieve a continuous process (Greenwell *et al.* 2009). For coagulation, the addition of aluminium to the culture solution is known to inhibit the growth of algae cells.

Recently, membrane filtration has been attracting attention as a new separation process (Greenwell *et al.* 2009). Because membrane filtration can perform solid–liquid separation without chemicals, it is actively used in the fields of food, pharmaceuticals, water treatment, and sewage disposal. Similarly, in the field of cell harvesting, membrane filtration is being investigated because of its

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high efficiency potential for mass cultivation and its reduced toxicity toward cells when the permeate medium is reused for the next cultivation. The major disadvantage of membrane filtration is 'membrane fouling', with many studies reporting a potential for significant fouling of the algae cells when using membrane filtration for the harvesting process (Castaing *et al*. 2011). Zhang reported that membrane fouling developed owing to the build-up of an algal cake layer and the adsorption of algal extracellular polysaccharides (EPS) (Zhang *et al*. 2010). EPS produced by algae are generally divided into two types; for example, solved-EPS (s-EPS) that are dissolved in the culture medium away from the cells and bound-EPS (b-EPS) that firmly adhere to the algae cell membrane. Previous research on membrane fouling has demonstrated that membrane fouling caused by b-EPS was different for different types of algae species (Chiou *et al*. 2010). Moreover, the trend of fouling is different between s-EPS and b-EPS (Qu *et al*. 2012). Several investigations have been conducted to improve the productivity of algae biomass fuel in the field of molecular biology, and various types of algae have been modified for greater productivity using genetic engineering procedures. Therefore, it is expected that there will be increasing numbers of mutants with an increasing variety of characteristics.

Although many studies have surveyed the fouling potential of a single species, there have been few studies reporting trends in membrane fouling affected by different species of algae. Moreover, to our limited knowledge, there has been no study showing the influence of algae strains on membrane fouling. In addition, little work is currently available in the published literature on methods for selecting suitable strains from the mutant strains available with respect to their low membrane-fouling potential.

In the present study, two strains of green algae were cultivated under the same conditions and filtered by a microfiltration membrane. The influence of the EPS characteristics on the performance of membrane filtration was investigated by comparing the EPS characteristics of two green algae and the degree of fouling.

MATERIAL AND METHODS

Algae and culture

Two strains of green algae, for example, S1 and S2, are categorized as the same species of *Pseudo-coccomyxa ellipsoidea*. Ninety-nine percent homology between S1 and S2 was confirmed by 18S-rRNA analysis. These strains were collected from different hot spring waters in Japan and isolated in our laboratory. They were cultivated in an aseptic environment. These algae can grow in conditions having a pH from pH 3 to pH 4, which is a significant advantage in open cultivation. The pH of the medium was adjusted to 3.5 ± 0.5, and the algae were cultivated in a flask (1 L working volume) for 14 days at approximately 23 °C under fluorescent lighting of 120 $\mu E/s/m^2$ for 24 hours. Both strains were cultured in a medium containing 6.3 mg/L of nitrogen and 0.8 mg/L of phosphorus. During cultivation, carbon dioxide enriched air (1%- CO_2 v/v) continuously aerated the 1 L flask via a form plug.

Membrane filtration system

The 3 L culture solution of the strain was filtered by a constant flux. Dead-end filtration experiments were carried out under conditions avoiding the occurrence of bubbling and shear stress by cross-flow around the membrane surface. Therefore, sediments on the membrane surface were considered to be cancelled only by backwashing.

Hollow fiber membranes (PVDF; Asahi Kasei) were used for the membrane module, having 98 cm^2 of membrane area with pores of 0.1 μm. Inner diameter, outer diameter, and pure water permeability were 6.4 ± 0.1 mm, 1.24 ± 0.1 mm and 0.03 m/day/kPa, respectively. The permeate flux was set at 0.4 m/day lower than the maximum operation flux, which was obtained under the selected operation schedule (i.e., 10-minute filtration and 15-second backwashing); that is, the flux increased TMP up to 5 kPa in 5 min. Based on pre-examination under various flux conditions, we identified that the maximum TMP increase rate was 5 kPa/min to achieve 10 times the concentration process. Backwashing was performed every 10 min for 15 s during the filtration. The flux of the backwash was set up at one-and-a-half times the permeate flux. The filtration ended when the concentration rate of the culture solution reached 10 times the starting concentration or when the TMP showed 90 kPa. After the membrane filtration experiments, the membrane surface was washed with water. The fouling that was not cancelled by this operation was defined as reversible fouling. In addition, the fouling that was only canceled by chemical washing using NaOH (pH 12) for 24 hours was defined as irreversible fouling

Each filtration resistance value was defined with the following expressions:

$$J = \Delta P/\mu(\mathrm{Rm} + \mathrm{Rre} + \mathrm{Rir}).$$

J: Membrane filtration flux (m/day), P: Membrane pressure, μ: viscosity of water, Rm: Membrane resistance, Rre: Reversible fouling resistance, Rir: Irreversible fouling resistance.

Analytical methods

To evaluate the specific growth rates of S1 and S2, a UV spectrophotometer (UV-1800; SHIMADZU) was used at 750 nm. Total nitrogen (TN), total organic carbon (TOC), and dissolved organic carbon (DOC) were measured with a TOC analyser (TOC-L; SHIMADZU). DOC was defined as the organic carbon from permeation that was filtered from the culture solution through a membrane filter with a pore size of 0.45 μm (hydrophilic PTFE; ADVANTEC). Particulate organic carbon (POC) was calculated by subtracting DOC from TOC, which means POC showed organic matter over 0.45 μm, like algal cells. The molecular weight distribution of EPS was measured by a LC-OCD (liquid chromatography–organic carbon detection) equipped with a UV spectrophotometer and a TOC analyser. Samples used for the LC-OCD measurement were adjusted to 2.5 ± 0.5 mg-C/L and filtered through the membrane filter with a pore size of 0.45 μm (hydrophilic PTFE; ADVANTEC). The surface charge of algal cells was detected by a zeta potential meter (ZEECOM; Microtec Nichion). The s-EPS solutions of S1 and S2 were obtained by centrifuging the culture solution at 3,000 g under 4 °C for 15 min with a high speed refrigerated centrifuge (CF16RX2; HITACHI) and filtering the supernatant through a 0.45 μm membrane filter (hydrophilic PTFE; ADVANTEC). The cells deposited in the bottom of centrifuge tube were collected and resuspended in phosphate buffer saline. Then, a cation exchange resin (DOWEX Marathon C; Sigma-Aldrich) was added to a suspended solution and the solution was stirred at 800 rpm under 4 °C for 4 hours. The b-EPS solutions of S1 and S2 were obtained by centrifuging the solutions at 12,000 g and under 4 °C for 15 min and subsequently filtering the supernatant with a 0.45 μm membrane filter (hydrophilic PTFE; ADVANTEC) (Wingender *et al.* 1999).

RESULTS AND DISCUSSION

Growth and characteristics of EPS of S1 and S2

The growth curve of S1 and S2 is shown in Figure 1. There was no difference in the trend of growth curve between S1 and S2. A maximum specific growth rate for S1 and S2 was 4.2 and 4.3 g/m^2/day, respectively, and the maximum growth volume of S1 and S2 was identical at 1.4 /cm.

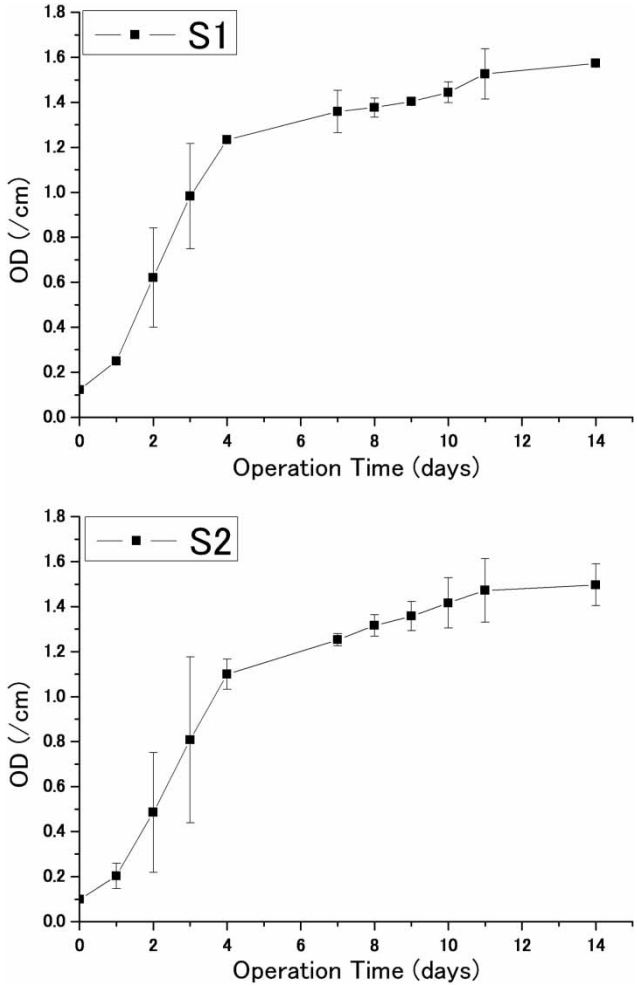

Figure 1 | Growth curve of S1 and S2.

These results indicate that the strains used in our present study have similar growth properties.

Table 1 shows the characteristics of the DOC in the culture solution containing algal cells, S1 and S2. As clearly shown in Table 1, a large difference in the value of DOC was seen between the two strains, although the value of OD (cell number) was almost identical. These results clearly showed that the amount of EPS produced by one cell of S1 and S2 was significantly different. S2 contained a greater amount of lipid substances than S1, and we confirmed different photosynthesis abilities between S1 and S2. Thus, we assumed that the respective metabolisms of algae cells, including photosynthesis ability, carbon fixation ability, carbon-releasing ability and breathing quantity, affected the production of DOC. Furthermore, the rate of nitrogen to carbon for S1 was twice that for S2, which indicates a difference in organic composition of the S1 and S2 culture

Table 1 | Characteristics of dissolved organic matter in culture solution and algae cells

Dissolved organic matter in culture solution	S1	S2
OD (/cm)	1.6 ± 0.2	1.6 ± 0.2
DOC (mg-C/L)	247.8 ± 57.3	31.2 ± 3.5
Algal cells	S1	S2
Dry weight (mg/L)	471.3 ± 78.9	462.5 ± 10.0
POC (mg-C/L)	167.2 ± 43.2	164.1 ± 24.7
N/C (%)	3.6 ± 0.9	1.3 ± 0.9
Oil contents (%)	32 ± 1	40 ± 2
Zeta potential (mV)	7.37 ± 5.3	15.5 ± 7.7

solutions. The average zeta potential of S1 and S2 was 7.37 and 15.5 mV, respectively, which suggests different organic compositions on the surface of S1 and S2 algal cells. The difference in EPS characteristics was probably caused by the difference in metabolisms of the S1 and S2 strains. Considering that the homology of S1 and S2 was more than 99%, even a small difference in genes was found to significantly affect the amount and quality of EPS. The following discussion will address how EPS characteristics influenced membrane filtration.

Figure 2 summarizes the results of the membrane filtration measurements of S1 and S2. The trigger fluxes for S1 and S2 were 2.6 and 3.2 m/day, respectively. The permeate flux was set at 2.2 and 1.0 m/day. The TMP of S1 showed a sharp rise as operation time increased, whereas the TMP of S2 was almost steady throughout. The differences in the TMP trends of S1 and S2 were considered to be a consequence of the differences in their EPS characteristics. The fouling ratio between the reversible and the irreversible fouling is shown in Figure 3, which shows that the reversible fouling between S1 and S2 was significantly different.

The discussion here will focus on the influence of EPS on irreversible and reversible fouling. First, we focus on the influence of EPS on the irreversible fouling. Table 2 shows the characteristics of the organic matter that caused the irreversible fouling of S1 and S2. The rates for carbon, nitrogen, carbohydrate, and protein were approximately the same, which supports the finding that there was no difference in the degree of the irreversible fouling between S1 and S2.

The components of organic matter that caused the irreversible fouling of S1 and S2 are shown in Figure 4. This shows that the carbohydrate-like substances (O–H; 3400–3300 and C–O; 1100–1000) and the lipid-like substances (CH3; 2960, COOR; 1740) (Leenher 2009) caused

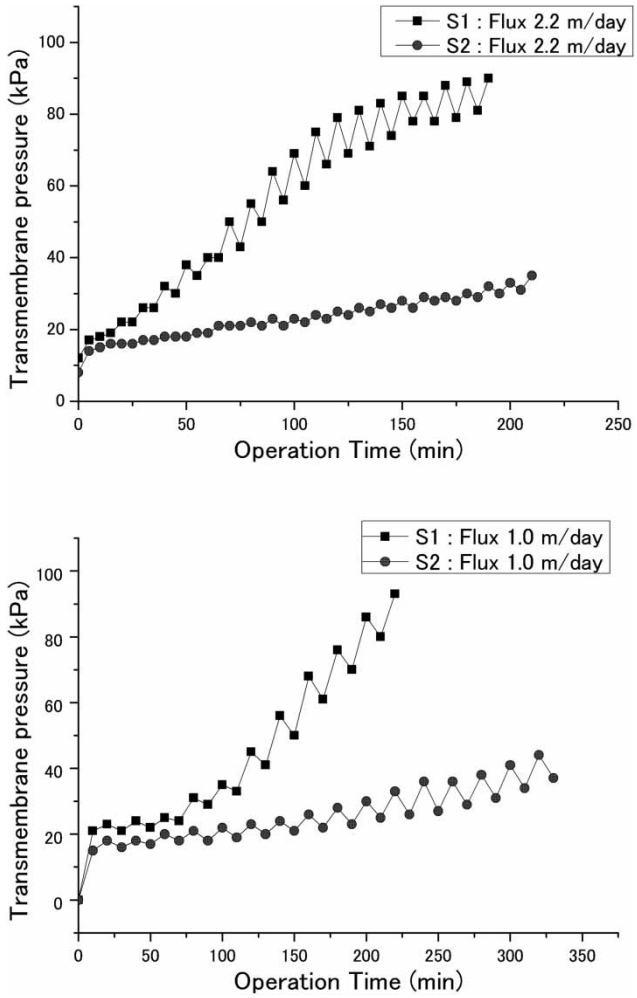

Figure 2 | Changes in transmembrane pressure.

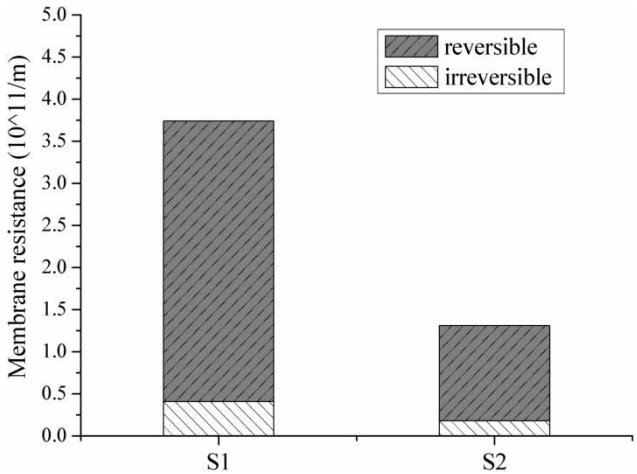

Figure 3 | The rate of the reversible fouling and the irreversible fouling of S1 and S2.

Table 2 | Concentration of EPS that caused irreversible fouling ($n = 2$)

	S1	S2
Carbon (mg-C)	1.84 ± 0.7	2.18 ± 0.22
N/C (%)	11.6 ± 5.61	6.45 ± 2.95
Carbohydrate (mg-C)	1.66 ± 0.94	1.22 ± 0.99
Protein (mg-C)	0.62 ± 0.21	0.23 ± 0.18

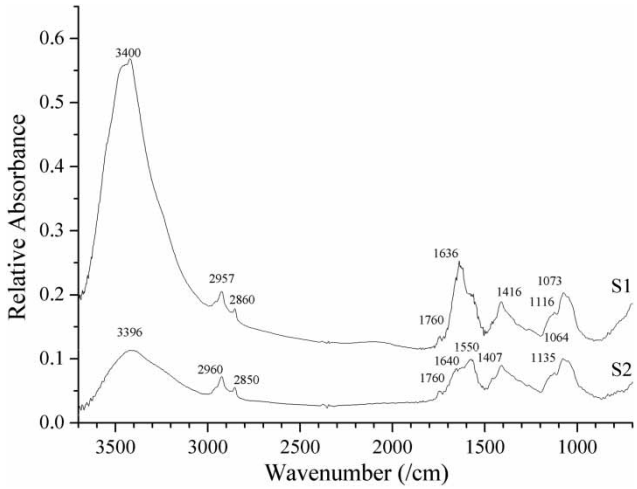

Figure 4 | Fourier transform infrared spectroscopy (FT-IR) spectra of EPS that caused irreversible fouling (upper line: S1, lower line: S2).

irreversible fouling in both operations. It could be stated that the foulants of S1 and S2 had the same components. Therefore, the important findings in this study are that the quantity and quality of organic matter that cause the irreversible fouling of S1 and S2 bear no relation to the differences in EPS.

Next, we focus on the influence of EPS on reversible fouling.

In this filtration experiment, sediments on the membrane surface were considered to be cancelled only by backwashing during operation. At the end of every filtration experiment, we washed the membrane surface with water. The fouling that was cancelled by those physical washings was defined as reversible fouling, while that cancelled only by chemical washing with NaOH was defined as irreversible fouling.

Since we cultivated the algae aseptically, the contamination of other microorganisms was not found, and thereby, the suspended solids (SS) in the culture medium were expected to result only from algae cells. This assumption has been verified by microscopic observation. Thus, the rejection of particles with 0.1 μm or larger diameter expected for the used membrane indicated that the SS components in the cake layer were algae cell bodies. Taken together, it was suggested that algae cells attached on membrane surfaces were not cancelled by backwashing, resulting in the increase in TMP during the operation.

Furthermore, since OD and filtration flux were maintained at the same degree in this study, we speculated that the different trend of reversible fouling between S1 and S2 was caused by differences in physico-chemical characteristics of the algae cells (e.g., the adhesivity of the algae cells on the membrane, and the quality of b-EPS surrounding the cells).

The characteristics of b-EPS in S1 and S2 are shown in Table 3, where a difference in the value of specific ultraviolet absorption (SUVA) and the ratio of nitrogen to carbon is shown, although the value of TOC was almost identical. This result means that there was no difference in the quantity of b-EPS in S1 and S2; however, the quality of b-EPS components in S1 and S2 was different.

Figure 5 summarizes the molecular weight distribution of b-EPS in S1 and S2. The upper line and lower line in Figure 5 show the molecular weight distribution of organic carbon and nitrogen, respectively. The detection of UV254 nm is plotted against the retention time in the middle line. As clearly shown in Figure 5, a large difference is seen in the peak height of biopolymers located at about 1,000,000 Da of the molecular weight distribution (at 30 min of retention time). Although composition and structure of the biopolymers are diverse, most biopolymers are said to be composed of carbohydrate-like and protein-like substances with a molecular size from 100,000 to 2,000,000 Da. As the hydrophobic substances were strongly adsorbed onto the beads in the column, all the peaks – including biopolymer peaks detectable on LC-OCD chromatograms – were attributed to hydrophilic substances. The detection of biopolymers in S1 coincides with the lower value of SUVA in b-EPS in S1 than that in S2 because biopolymers are hydrophilic and cause membrane fouling.

Table 3 | Characteristics of b-EPS of S1 and S2 ($n = 3$)

	S1	S2
Carbon (mg-C)	34.4 ± 2.6	44.4 ± 12.9
N/C (%)	1.8 ± 0.35	1.0 ± 0.3
Carbohydrate (mg-C)	5.8 ± 2.5	7.8 ± 1.9
Protein (mg-C)	0.6 ± 0.1	0.4 ± 0.1
SUVA (mg/cm/L)	4.4 ± 0.2	4.5 ± 1.9

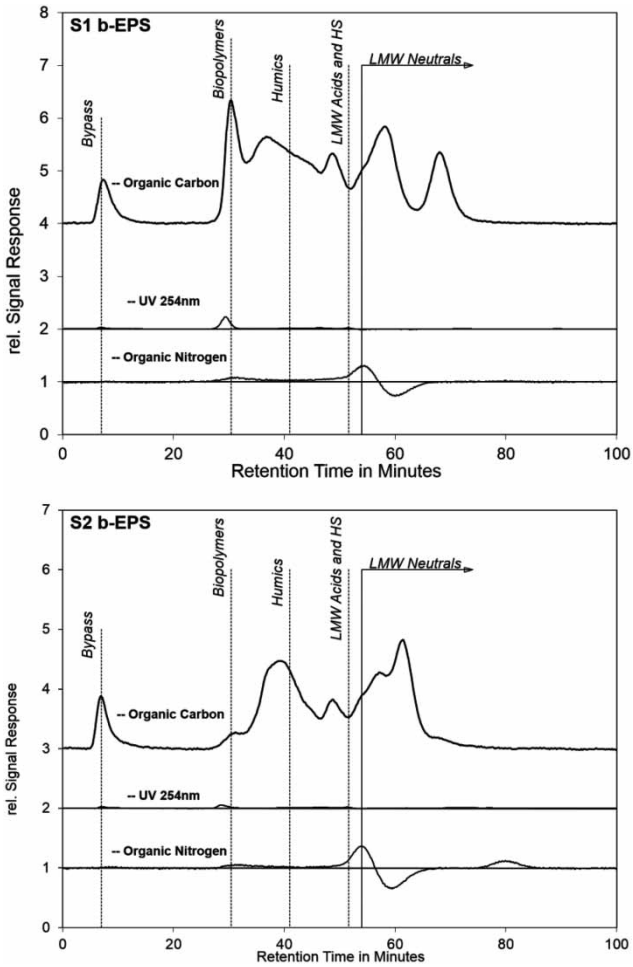

Figure 5 | Molecular weight distribution of b-EPS in S1 and S2.

Therefore, it is proposed that the difference in the amount of biopolymers of b-EPS in S1 and S2 may account for the difference in the amount of reversible fouling between S1 and S2.

CONCLUSION

In the present study, two strains of green algae named S1 and S2, categorized as the same species of *Pseudo-coccomyxa ellipsoidea* but showing 99% homology, were cultivated under the same conditions and filtrated by a microfiltration membrane. There was no difference in the specific growth rate between the S1 and S2 strains; however, large differences were seen in the amount and quality of EPS between S1 and S2. When the S1 and S2 strains were filtered with a membrane, the trend in the increase in transmembrane pressure (TMP) was quite different. The filtration of the S1 strain showed a rapid increase in TMP, whereas the TMP of the filtration of S2 strain did not increase at all during the operation. This clearly demonstrated that the characteristics of each strain affect the development of membrane fouling. On the basis of the detailed characterization of s-EPS and b-EPS, it was clarified that s-EPS mainly contributed to irreversible fouling for both operations and the biopolymer-like organic matter contained in b-EPS mainly contributed to reversible fouling. The role of biopolymers was suggested as being important in reversible fouling development, and further research on the relationship of EPS characteristics of different algae strains and fouling development are necessary.

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