


Fouling potential of lipopolysaccharides released at low temperatures in MBRs

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ABSTRACT

Sludge filterability in membrane bioreactors (MBRs) fluctuates and affects membrane fouling. Therefore, understanding the reasons for the fluctuations of sludge filterability is important for the efficient operation of MBRs. In this study, a pilot-scale MBR treating municipal wastewater was operated for about 600 days and the variations in sludge filterability were continuously monitored by batch-filtration experiments using the same membranes as in the MBR. To investigate the reasons for the deterioration of sludge filterability, constituents in sludge supernatant were intensively monitored, and the correlations with sludge filterability were determined. The concentration of lipopolysaccharides (LPS) in sludge supernatant exhibited significantly higher correlation with sludge filterability than did conventional indexes (i.e. polysaccharides and proteins). Size fractions affecting MBR sludge filterability were also investigated, and it was suggested that colloidal LPS deteriorated MBR sludge filterability. Based on the long-term operation of the MBR, increase in colloidal LPS under low temperatures of the mixed liquor suspension was a key factor in the deterioration of sludge filterability. The impact of LPS increasing under low temperatures should be investigated by operating bench-scale MBRs fed with synthetic wastewater in controlled conditions.

Key words | lipopolysaccharides (LPS), membrane fouling, wastewater treatment

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INTRODUCTION

Membrane bioreactors (MBRs) are good alternative wastewater treatment systems to conventional activated sludge systems (CASs) since high quality effluent with a small footprint and easy operation can be achieved. However, membrane fouling is one of the major drawbacks for the wider application of MBRs because it increases operation and maintenance costs (Le-Clech *et al.* 2006; Drews 2010; Krzeminski *et al.* 2017; Meng *et al.* 2017). It is well known that sludge filterability in an MBR varies significantly over long-term operation and affects the evolution of membrane fouling. For the efficient operation of MBRs, understanding the reasons for the fluctuation of sludge filterability, and appropriate assessment of sludge filterability are important (Van den Broeck *et al.* 2011).

In previous studies on membrane fouling in MBRs, the influence of organic matter in sludge supernatant on membrane fouling have been pointed out (Rosenberger *et al.* 2005; Lyko *et al.* 2007; Sun *et al.* 2014). Zhang *et al.* (2019) recently reported that fouling rate was related to the fouling potential of soluble microbial products (SMP) in MBRs.

Also, Christensen *et al.* (2018) concluded that the colloidal fraction in sludge supernatant is the most important in membrane fouling in MBRs. For these reasons, the constituents of sludge supernatant were focused on in this study. In our previous study, lipopolysaccharides (LPS) originating from the cell walls of Gram-negative bacteria were identified as key components in MBR fouling (Kimura *et al.* 2015). It was also suggested that LPS in mixed liquor suspension have high fouling potential (Kimura *et al.* 2019). Thus, variations in the concentration of LPS in MBR sludge supernatant were also monitored in this study, and the relationship with sludge filterability was investigated. To the best of our knowledge, this is the first report to show the results of continuous monitoring of LPS in the sludge supernatant of an MBR.

In this study, a pilot-scale MBR treating municipal wastewater was continuously operated for about 600 days, and the sludge filterability was monitored. Also, key factors affecting MBR sludge filterability were intensively investigated.

MATERIAL AND METHODS

Operation of a pilot-scale MBR

All sludge samples were obtained from a pilot-scale MBR (effective volume: 450 L) installed at an existing wastewater treatment plant (Soseigawa Wastewater Treatment Center, Sapporo, Japan). This plant is connected to a combined sewer system, and the influent to the primary sedimentation basin of the plant was fed to the MBR after being sieved by a bar screen (openings: 1 mm). The flat sheet membranes in the MBR were made from PVDF with a nominal pore size of 0.1 μm (Toray, Japan). Total membrane surface area was 5.44 m^2 . The solids retention time (SRT) and the hydraulic retention time (HRT) during the operation were fixed at 30 days and 6.1 hours, respectively. Sludge sampling was initiated after acclimatization of the sludge was confirmed by an operation for 90 days (triple the SRT).

Batch filtration experiments

To assess the MBR sludge filterability, batch filtration of the sludge was carried out using a commercially available filtration cell (Advantec Toyo, Tokyo, Japan). Membranes used in the batch-filtration tests were the same as the ones in the pilot-scale MBR. Filtration was conducted under a constant pressure of 15 kPa for 30 min with stirring in the cell (300 rpm). Based on the recorded flux, the filtration resistance caused by the filtration of samples was determined by Darcy's law:

$$R = \frac{\Delta P}{\mu \cdot J} \quad (1)$$

where R is the membrane filtration resistance (m^{-1}), J is the permeate flux ($\text{m}^3/\text{m}^2/\text{s}$), ΔP is the trans-membrane pressure (Pa) and μ is the viscosity of the permeate (Pa·s). The membrane filtration resistance can be divided into two types of resistance as follows:

$$R = R_m + R_f \quad (2)$$

where R_m is the membrane intrinsic resistance (m^{-1}) and R_f is the resistance caused by membrane fouling (m^{-1}). In this study, R_f was used as an index of sludge filterability.

Analytical methods

Sludge samples obtained from the pilot-scale MBR were centrifuged (3,000 g, 5 min) and the supernatant was thought to

contain colloidal matter and dissolved matter (Wu & Huang 2009). The supernatant was filtered using a mixed cellulosic ester membrane (Advantec Toyo, Tokyo, Japan) with a pore size of 0.45 μm and the filtrate was defined as dissolved fractions. The concentration of polysaccharides and proteins were determined by using the phenol-sulfuric acid method (Dubois *et al.* 1956) and the Lowry method (Lowry *et al.* 1951), respectively. Glucose and bovine serum albumin (BSA) were used as standards for the measurement of polysaccharides and proteins, respectively. The concentration of LPS was determined using an endotoxin assay kit (Wako Pure chemical industry, Osaka, Japan). The level of LPS was presented as endotoxin units per millilitre (EU/mL).

RESULTS AND DISCUSSION

Seasonal variations of MBR sludge filterability

Figure 1 shows the changes in filtration resistance caused by the batch-filtration experiments of MBR sludge and the temperature of the mixed liquor suspension in the pilot-scale MBR. It can be seen in the figure that sludge filterability significantly fluctuated over the long-term operation of the pilot-scale MBR. In the early stages, sludge filterability was high and stable. However, filtration resistance started to rise around day 50 of the operation. Filtration resistance then started to decrease after about 90 days of the operation and was almost stable from days 200 to 400 of the operation. The reason for the decline in the filtration resistance was not clear, although changes in sludge supernatants are likely to have been responsible. Filtration resistance increased again after about 400 days of the operation. It was obvious that sludge filterability deteriorated when the temperature of the mixed liquor suspension was low. Previous studies have shown that severe membrane fouling is observed under low temperature (Miyoshi *et al.* 2009; van den Brink *et al.* 2011). Our results obtained in this study were in accordance with those previous studies (i.e. deterioration of sludge filterability under low temperature).

Relationships between constituents in sludge supernatant and sludge filterability

Figure 2 shows the correlations between the concentrations of constituents in the sludge supernatant and filtration resistance caused by batch filtration of MBR sludge. In this study, the non-parametric Spearman's rank correlation was performed since the distributions of the data points may not

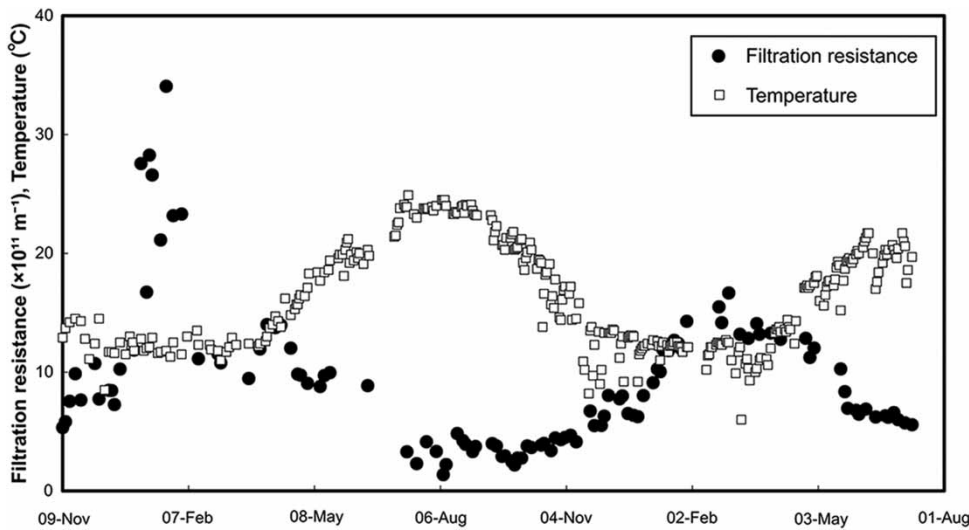


Figure 1 | Changes in filtration resistance caused by batch-filtration experiments of MBR sludge and temperature of mixed liquor suspension in the pilot-scale MBR.

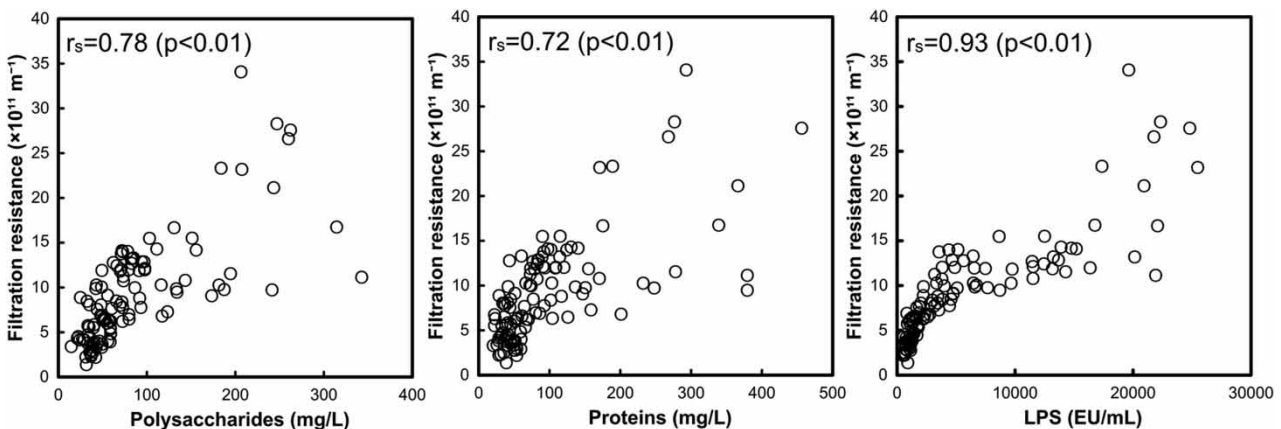


Figure 2 | Correlations between constituents in sludge supernatant and filtration resistance recorded in the batch-filtration experiments of MBR sludge.

have been normally distributed (Delrue *et al.* 2011). The values of the correlation coefficients (r_s) were tested for their statistical significances, and a p -value of 0.05 was used as a significance threshold. As discussed in previous studies (Rosenberger *et al.* 2006, 2005; Wu & Huang 2009), the concentrations of organic matter were correlated with sludge filterability. It should be emphasized that LPS in the sludge supernatant exhibited a more significant correlation with sludge filterability than the polysaccharides and proteins. Recent studies have indicated the importance of focusing on specific polysaccharides/proteins (Kimura *et al.* 2012; Miyoshi *et al.* 2012; Le & Stuckey 2016). The results obtained in this study might be in accordance with their postulations. It should also be noted that the measurement of LPS is much simpler and quicker than conventional

colorimetric methods. Thus, monitoring LPS is recommended for the assessment of MBR sludge filterability.

Size fractions affecting MBR sludge filterability

Figure 3 shows the correlations between the concentrations of dissolved matter in sludge supernatant and the filtration resistance recorded in the batch filtration of sludge samples. The correlations between dissolved matter and sludge filterability were significantly weaker than those between supernatant and sludge filterability. This implies that the constituents in sludge supernatant retained by membranes with a pore size of $0.45\ \mu\text{m}$ (i.e. colloidal matter) significantly affected MBR sludge filterability. Therefore, the concentrations of colloidal constituents were determined

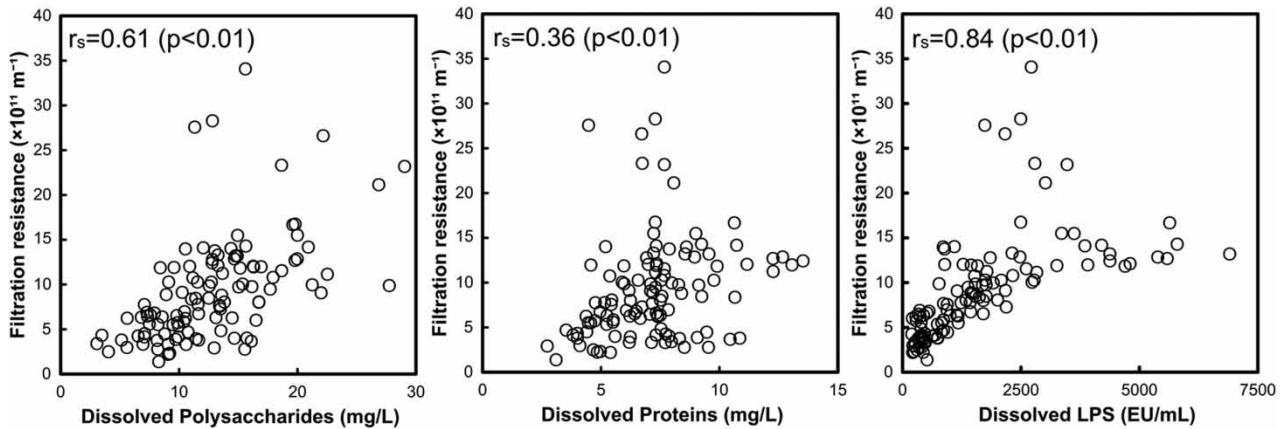


Figure 3 | Correlations between dissolved constituents in sludge supernatant and filtration resistance recorded in the batch-filtration experiments of MBR sludge.

by subtracting the dissolved concentration from the total concentration in the sludge supernatant, and the correlations between the colloidal constituents and sludge filterability are shown in Figure 4. It is clear that the colloidal constituents were more strongly correlated with sludge filterability than the dissolved constituents. It was also suggested that colloidal LPS were key players affecting MBR sludge filterability. Recently, it was reported that the accumulation of low biodegradable organic colloids in the supernatant of an MBR worsened sludge filterability (Scholes *et al.* 2019). These results suggest that sludge filterability can be controlled by preventing the accumulation of problematic colloids in mixed liquor suspension.

Factors affecting increase in colloidal LPS

As discussed above, colloidal LPS might be important fractions affecting MBR sludge filterability. Therefore, factors affecting increases in colloidal LPS were also investigated

in this study. Figure 5 shows the relationship between the temperature of the mixed liquor in the pilot-scale MBR and the concentration of colloidal LPS. It is clear that the level of colloidal LPS rose when the temperature was low ($<15\text{ }^{\circ}\text{C}$). This result suggested that a stressful condition (i.e., low temperature) might have promoted a release of LPS from the biomass. The released LPS were probably in dissolved form, and they change to the colloidal form under certain conditions. It was reported that biodegradation of organic matter by microorganisms decreased at low temperatures (Krzeminski *et al.* 2012). Degradation of colloidal LPS might also be affected at low temperatures. In this study, unfortunately, changes in another factor (i.e. the ion balance in the feed wastewater) were observed at around the same period of the operation of the MBR (data not shown). It was uncontrollable in this study because real municipal wastewater was fed to the MBR. To confirm the effect of low temperature on an increase in the concentration of colloidal LPS, experiments under controlled

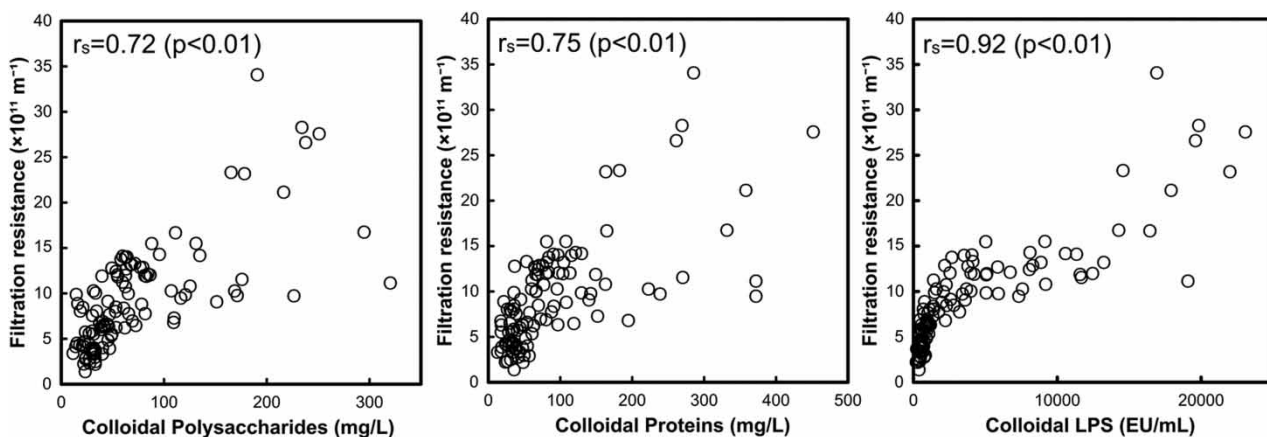


Figure 4 | Correlations between colloidal constituents in sludge supernatant and filtration resistance recorded in the batch-filtration experiments of MBR sludge.

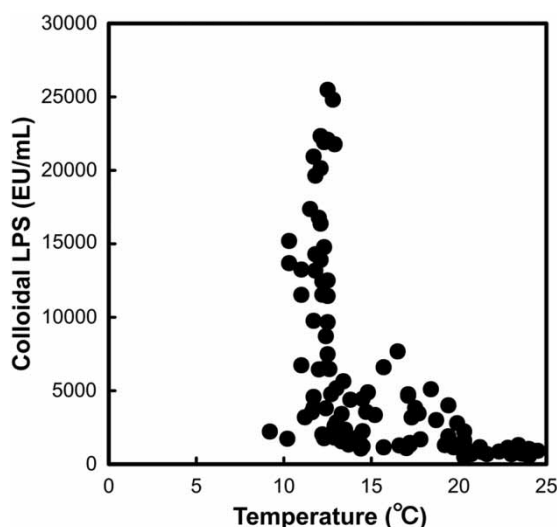


Figure 5 | Relationship between temperature of mixed liquor suspension in the pilot-scale MBR and colloidal LPS in sludge supernatant.

conditions should be conducted. Therefore, the impact of temperature on an increase in colloidal LPS are now being investigated by operating bench-scale MBRs fed with synthetic wastewater under controlled conditions.

CONCLUSIONS

In this study, a pilot-scale MBR treating municipal wastewater was operated for about 600 days, and variations in sludge filterability were continuously monitored. Sludge filterability significantly fluctuated over the long-term operation of the MBR and deteriorated under low temperature. Key factors affecting MBR sludge filterability were also investigated, focusing on the constituents in the sludge supernatant. It was suggested that colloidal matter had a significant impact on MBR sludge filterability. Colloidal LPS exhibited a stronger correlation with sludge filterability than did polysaccharides and proteins determined by conventional colorimetric methods. The level of colloidal LPS rose at low temperatures of the mixed liquor in the pilot-scale MBR. The effect of low temperature on the increase in colloidal LPS is now being investigated by operating bench-scale MBRs fed with synthetic wastewater under controlled conditions.

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