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Retention of soluble microbial products in submerged membrane bioreactors

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ABSTRACT

The retention of soluble microbial products (SMP) is significant for the operation of membrane bioreactors (MBRs) from the aspect of both effluent quality and membrane fouling. Constant flux stirred dead-end filtration of a MBR sludge supernatant was conducted. The SMP retention by the membrane is dynamic rather than consistent during filtration. Polysaccharides and proteins are the major components of SMP that are most easily retained by the membrane. The framework of the gel layer is mainly formed from polysaccharides, which controls its permeability. The SMP proteins appear to be trapped in the gel layer by steric and/or adsorptive effects but play little structural role. The underlying mechanism for adsorptive retention may arise from intermolecular electrostatic attraction or chemical complexation involving metal ions.

Keywords: Activated sludge; Size fractionation; Free EPS; Wastewater treatment; Proteinase

1. Introduction

Since its introduction in the late 1980s [1], the submerged membrane bioreactor (MBR) has attracted considerable attention with regard to its potential for application in wastewater treatment and water reclamation [2,3] in view of its considerable advantages over conventional processes [4]. The process will become even more attractive in future with further reduction in membrane prices and increasing understanding of the factors contributing to and means of overcoming membrane fouling, commonly believed to be the one major obstacle to wider application of MBR technology [5]. Membrane fouling is defined as any deposition of inorganic and organic matter and biomass onto the membrane [6]. At first glance, membrane fouling would appear to be totally detrimental because of the associated reduction in membrane permeability. However, membrane fouling is beneficial to the improvement of the effluent quality as a result of the fouling layer acting as a secondary barrier. This is mainly the reason that the sludge supernatant inside the MBR has often been reported to exhibit a consistently higher organic content than the MBR effluent [3,7,8].

Sub-critical flux operation with intense aeration that brings about high shear intensity and membrane vibration [9] is commonly employed to suppress biomass deposition or cake formation on the membrane. Thus, soluble and colloidal substances have been shown [10–12] to contribute more to membrane fouling in MBR than does the sludge particulates. Both the soluble and colloidal organic constituents are generally included in the broadly defined category of ''soluble microbial products'' (SMP) [13] (or soluble extracellular polymeric substances [5]) although, strictly speaking, SMP are only those soluble organics of biological origin. SMP are far from homogenous in either

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chemical (such as components and functional groups) or physical (such as size, shape and density) properties. Various SMP fractions may have different contributions to membrane fouling. As such, SMP fractionation is necessary for a better understanding of membrane fouling. Indeed, Rosenberger et al. [14] found that the membrane fouling rate could be linearly related with the polysaccharides content of SMP while Liang et al. [15] found that the neutral hydrophilic fraction appeared to be responsible for membrane fouling though the majority of the SMP components were hydrophobic. Other investigators have fractionated the SMP by size [14,16,17] and examined the implications to membrane fouling. Larger SMPs were usually considered more responsible for membrane fouling.

This study is an attempt to clarify the significance of SMP retention to MBR operation in terms of both effluent quality and membrane fouling. Constant flux stirred dead-end filtration of a MBR sludge supernatant was employed for a better control of the experimental conditions with particular attention given to the role of proteins and polysaccharides in membrane fouling.

2. Materials and methods

2.1. Filtration set-up

A dead-end filtration cell was employed for both constant flux and constant pressure filtrations. The acrylic filtration cell had a volume of 200 mL and effective filtration area of 1.96×10^{-3} m^2. A magnetic stirring bar identical to that used in the Amicon 8400 (Millipore) unit was installed in the filtration cell leaving a gap of 3.0 mm to the membrane's upper surface. The rotation speed of the magnetic bar was set to 120 rpm when shear application was required otherwise the magnetic bar was removed. For operation in constant flux mode, a peristaltic pump (Masterflex, Cole-Parmer) was connected to the outlet of the filtration cell with the inlet open to the atmosphere. A pressure transducer (Universal CB1020, Labom) was installed between the filtration cell and the pump in order to monitor the variation in applied pressure during filtration. The pressure profile was logged as a function of operation time using Labview (National Instruments) software. The filtration production rate was logged by an electronic balance that was connected to a personal computer. The production rate was used to check the constancy of the filtration flux, which may drop with the increase of the required driving pressure. In this study, the trans-membrane pressure (TMP) was normalized using an identical flux (0.75 m/d) by

$$\Delta P_n = \Delta P_m \frac{J_n}{J_m}, \quad (1)$$

where ΔP is TMP, J is the filtration flux, and subscript m and n represent measured and normalized values, respectively. For operation in constant pressure mode, the filtration cell was pressurized by compressed air to the pre-set value that was controlled by a pressure controller (P-602C, Bronkhorst) which ensured a constant and precise applied pressure. The production rate was again recorded using a data-logged electronic balance.

2.2. Filtration of MBR sludge supernatant

The MBR was of a lab-scale pre-denitrification process including an anoxic (6 L), an aerobic (18 L) and a filtration compartment (6 L) separated by baffles. Two identical membrane modules of polyvinylidene fluoride (PVDF) hollow fibers (0.04 μm Zenon) were immersed vertically in the filtration zone which was intensively aerated by coarse bubbles for membrane cleaning. The effluent was drawn through the membrane modules by a suction pump (Masterflex, Cole-Parmer) in an intermittent mode (9 min on:1 min off). A concentrated organic feed and a concentrated inorganic feed prepared according to the basic recipe described in the Environmental Engineering Process Laboratory Manual of AEESP [18] were continuously pumped at the same rate into the reactor. Tap water, which was used for the dilution of the concentrated feeds, was supplied to the reactor at a rate which was automatically controlled by a solenoid valve connected with a water level sensor. The organics besides yeast extract in the feed were glucose and sodium acetate with a weight ratio of 1:2 that was set to maintain the pH of the sludge at or near neutrality. The hydraulic and sludge retention times were 8 h and 20 days, respectively. The mixed liquor suspended solid (MLSS) concentration was about 12 g/L corresponding to a F/M ratio of about 0.188 g-COD/g-SS/d. The supernatant was collected as the centrate after twice centrifuging at 3000 rpm for 10 min.

A fresh micro-filtration membrane disk of 0.45 μm pore size (Durapore, Millipore) was used for each filtration. Prior to each filtration, the membrane was fully ''wetted'' whereby Milli-Q water (Millipore) was filtered through the membrane in its unfouled state until a constant flux or a constant pressure was attained. Subsequently, 200 mL of supernatant was placed in the cell in readiness for the filtration experiment. The

filtration permeate was successively collected as samples of pre-set volumes which were utilized for the determination of total organic carbon (TOC) (and its protein, polysaccharide and humic contents) and average particle size. The measurement determined in each sample represents the average value within the period that was taken to collect the sample.

The effect of proteinase on membrane fouling rate was investigated by adding 0.1 mL Endopeptidase K solution (20 mg/mL, Qiagen GmbH) into the filtration cell filled with 200 mL supernatant. Constant flux filtration was initiated after overnight mixing of the dispersion. A control filtration was also conducted without proteinase addition.

2.3. Retention of protein by polysaccharide gel layer

The ability of a model polysaccharide gel layer formed from Ca-alginate dispersion to retain protein was investigated by using a model protein bovine serum albumin (BSA). The alginate dispersion was prepared by dissolving 1.00 g sodium alginate (Aldrich), 50 mmol NaCl, 2 mmol $NaHCO_3$ and 0.2 g NaN_3 into high-purity water (Milli-Q, Millipore) and making the final volume to 1.00 L. After overnight magnetic stirring of the dispersion, a further 3.78 mmol $CaCl_2$ was added into the dispersion followed by another overnight magnetic stirring. BSA dispersion was prepared by dissolving 1.0 g BSA (Sigma), 50 mmol NaCl, 2 mmol $NaHCO_3$ and 0.2 g NaN_3 in high-purity water (Milli-Q, Millipore) and making the final volume to 1.00 L.

The gel layer was firstly formed by dead-end filtration of the previously described Ca-alginate dispersion through a 0.22 μm membrane (Durapore, Millipore) at a fixed applied pressure of 12.5 kPa or 100 kPa without stirring. After about 40 mL permeate (which corresponds to a specific volume of about 2 cm) was collected, the filtration was terminated and the remaining feed dispersion was decanted. While the gel layer was kept intact on the membrane surface, about 50 mL BSA dispersion was placed in the filtration cell, which was then re-pressurized to the original pressure to commence the protein retention experiment. A 2 mL sample of permeate was collected each time the accumulated volume of permeate just exceeded 2 mL with this process being repeated 5–6 times. The BSA concentration in each sample was then determined by measuring the absorbance at 280 nm using a Cary 50Bio, Varian spectrometer. Using standard BSA solutions, BSA was found to have an extinction coefficient of 0.5791 L/g/cm at 280 nm. The concentration determined in each sample represents the average concentration of the BSA that passed through the gel layer within the period between two successive sample times.

2.4. Analytical items

Total organic carbon was measured by a TOC analyzer (TOC-5000A, Shimadzu) using the combustion-infrared method. The proteins and humic substances were determined by a UV–Vis spectrophotometer (Cary 50Bio, Varian) following the modified Lowry method [19] using BSA and humic acid as the standards, respectively. The polysaccharide content was measured according to the phenol method [20] using glucose as the standard. Particle size distribution (PSD) was determined using a Malvern Nano-ZS dynamic laser scattering (DLS) instrument. The DLS measures the Brownian diffusion constant which is then interpreted as the hydrodynamic radius of a diffusing sphere via the Stokes-Einstein equation (Jones, 2002). The average particle size was calculated to be the median (50%) of the cumulative distribution of the intensity.

The deposition layer formed from filtration was analyzed by scanning electron microscopy (SEM) imaging performed on cryogenically fixed specimens. The detailed manner of sample preparation is described elsewhere [21]. In brief, a strip of the fouled membrane of width of about 2 mm and length of about 1 cm was cut and fixed to a holder and immersed into a liquid nitrogen bath for about 15 s. The sample on the holder was then snapped such that the cross-section of the cake could be examined by SEM imaging. All of the SEM images were taken within a few minutes of freezing on a normal stage. On completion, the specimen was still in a solid state but with the top surface showing some signs of heat damage.

3. Results and discussion

3.1. Relevance of membrane fouling to SMP retention

The membrane modules in the MBR were vigorously bubbled to restrict the deposition of sludge flocs onto the membrane surface thus attenuating membrane fouling. Indeed, little visible sludge could be found on the severely fouled membrane when the TMP was higher than 50 kPa. SMP are believed responsible for membrane fouling of the MBR. Batch constant flux stirred dead-end filtration of the supernatant showed that the SMP fouled the membrane irrespective of shear though the fouling propensity appeared to fluctuate with operation time (compare Figs. 1a, 2 and 3). That shear has little effect on fouling propensity is

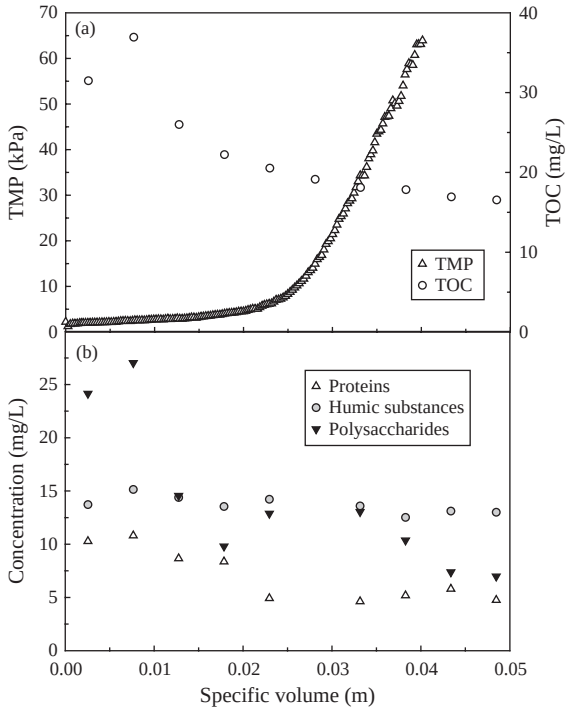

Fig. 1. Membrane fouling of constant flux (0.75 m/d) stirred dead-end filtration of the MBR supernatant indicated by (a) TMP increase and SMP retention by the membrane and (b) the retention of individual contents.

perhaps not surprising given that soluble or colloidal substances would be expected to be minimally affected by the applied shear. While the pore size of the membrane (0.45 μm) is considerably larger than the size of SMP in the supernatant, SMP presumably adsorbs initially to the membrane followed by SMP aggregation as the concentration of retained SMP near the membrane increases and, subsequently, gelation. Indeed, in a previous study [22], we showed that gel layer formation occurred soon after the commencement of filtration. The permeate TOC concentration profile (Fig. 1a) is a good indication of the transformation from adsorptive fouling to gel layer type fouling with the highest effluent TOC concentration indicating roughly the point that the transformation occurs. A comparison of the TOC profile and the TMP profile reveals that gel layer formation was the major fouling type during filtration of the supernatant (Fig. 1a).

Moreover, the permeate TOC was far from a constant (Fig. 1a) with the TOC decreasing continuously once gel layer type fouling began. Membrane fouling is closely related with SMP retention as a result of the formation of a gel layer. The gel layer is usually highly porous and compressible [21] which leads to an accelerated TMP increase during filtration (Fig. 1a) (compression induces a lowering in porosity with concomitant increase in specific resistance). The main constituents of SMP are proteins, polysaccharides and humic substances [16]. Thus, gel layer formation is the result of the interactions among these macromolecules under the conditions of the aqueous environment (such as pH, ionic strength and metal concentrations). Analysis of the concentration of these components in the permeate shows that the protein and polysaccharide concentrations followed the trend observed for TOC, while the humic substances concentration remained almost constant (Fig. 1b). This result suggests that proteins and polysaccharides were more likely involved in the gel layer formation and thus membrane fouling.

3.2. Relative significance of proteins and polysaccharides in membrane fouling

Given the fact that both polysaccharides and proteins were retained by the membrane to form a gel layer, it is possible that they both contribute to gel layer formation. However, the addition of proteinase (Endopeptidase K) was observed to have no effect on the membrane fouling rate (Fig. 2). Endopeptidase K is capable of catalyzing the cleavage of internal bonds in a polypeptide or protein. The protein concentration in the untreated supernatant was 17.0 ($\pm$2.3) mg/L BSA equivalent. After reacting with 10 mg/L proteinase added to the supernatant, those proteins were most likely cleaved into short segments of totally different properties. The proteinase itself is an exotic protein that is quite different in property to the native proteins. It is highly unlikely that the remnant proteins or peptides could function in a manner similar to the native proteins. As such, the proteinase addition result

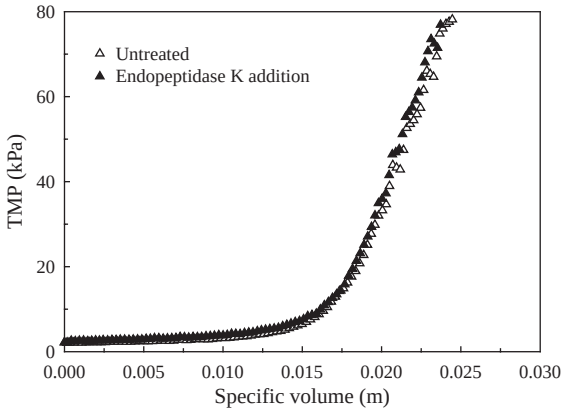

Fig. 2. Effect of proteinase (Endopeptidase K) addition on membrane fouling rate of constant flux (0.75 m/d) stirred dead-end filtration of the MBR supernatant.

strongly suggests that proteins are not involved structurally in gel layer formation.

Polysaccharides had a much higher concentration (69.4 ($\pm$4.0) mg/L) than proteins in the supernatant. The difference in the concentrations of polysaccharides and proteins between the supernatant and the filtration permeate (Fig. 1b) roughly reflects their contribution to the mass of the gel layer. Indeed, the concentration ratio of polysaccharides to proteins in a dispersion of SMP detached from the MBR membrane modules was about 4:1 (the detached SMPs were obtained by thoroughly rinsing the membrane modules with 150 mL Milli-Q water when they were severely fouled as indicated by a high (above 50 kPa) TMP followed by the removal of large particles using double centrifugation at 3000 rpm for 10 min). The large difference between the polysaccharide and protein contents in the gel layer supports the conclusion that polysaccharides are principally responsible for the framework of the gel layer. It has previously been found that the membrane fouling rate could be linearly related with the SMP polysaccharide content [14]. Indeed, a number of natural polysaccharides have been found to be capable of forming a gel under particular conditions [23].

3.3. Mechanism of protein retention

Although proteins may contribute little to the permeability of the fouling (gel) layer, the retention behavior is still important because it greatly affects the quality of the MBR effluent. Indeed, the ability to produce a high quality effluent is regarded as one of the important features of the MBR process. As mentioned above, the gel layer is usually highly compressible. The elevation in TMP that is required to maintain constant flux during filtration (Fig. 1a, 2 and 3) caused by membrane fouling would progressively compress the formed gel layer resulting in a lowered channel size for water passage. With the decrease of the channel size, it is expected that more and more colloidal particles including proteins may not be able to pass through the gel layer because of size exclusion. The profile of the average size of the permeate SMP shows this effect (Fig. 3). The relative constancy of the average size at the beginning of the filtration indicates that the extent of adsorption is influenced very little by particle size. When gel layer formation commenced however, the permeate SMP average size decreased quickly. Although it is not easy to continue to monitor the average size of macromolecules and colloids in the permeate because of the reduction in permeate TOC that occurs on continued filtration (Fig. 1a), further reduction of the average size as filtration progresses is expected.

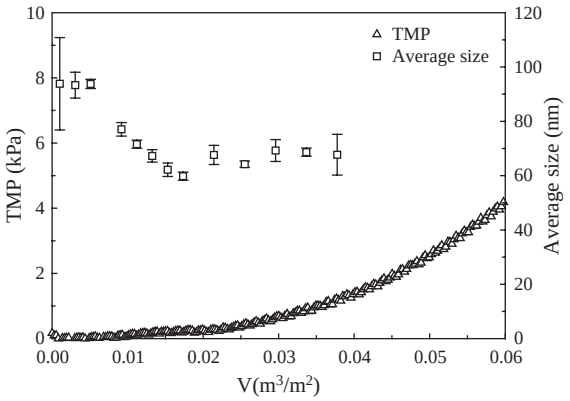

Fig. 3. Retention behavior by membrane during constant flux (0.75 m/d) stirred dead-end filtration of the MBR supernatant indicated by the change of the average size of the effluent SMP.

While gel formation certainly increases capture of SMP, some proteins are too small to be trapped by the gel layer or the membrane through size exclusion. Consider, for example, entrapment of BSA by a fouling layer formed by calcium-mediated alginate gelation. BSA has an average molecular weight of 66.7 kDa and a hydraulic radius of about 8.6 nm based on the DLS measurement. The calcium alginate gel layer, on the other hand, has significantly larger channel sizes for water passage than suggested by mathematical calculation [24] (Fig. 4a) (two possible reasons are the non-uniformity in channel size and the lack of connectivity of the transport pathways). However, the retention results of BSA by Ca-alginate gels show that some proportion of BSA could be removed from the effluent by gel layers formed by application of constant pressures of either 12.5 kPa or 100 kPa (Fig. 4b). A likely mechanism of retention is that of adsorption of BSA to the gel. As shown in Fig. 4b, little difference in retention ratios is observed between the 12.5 kPa and the 100 kPa gel layers at any given specific mass of BSA supplied to the gel layer ($m_{BSA}/m_{alginate}$). Furthermore, the fact that the retention ratio decreases with $m_{BSA}/m_{alginate}$ is supportive of adsorption being the primary mechanism for retention of BSA by the gel. If the gel layer is regarded as a thick adsorption column, then the breakthrough curve and the column uptake can be correlated by Thomas' approximation

$$\ln\left(\frac{C_0}{C}-1\right)=\frac{k_{TH}C_eM}{Q}-k_{TH}C_0t=\frac{k_{TH}M}{Q}(C_e-C_t), \quad (2)$$

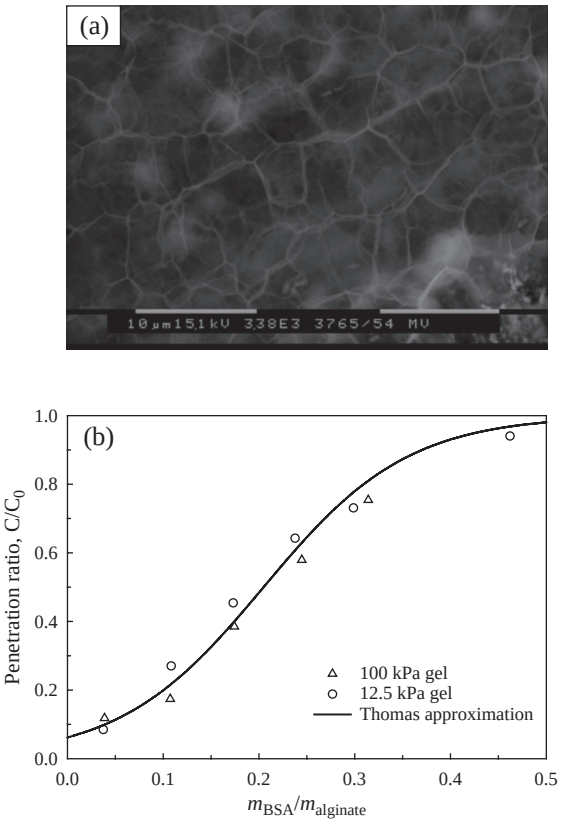

Fig. 4. Ca-alginate gel layer (a) shown by SEM imaging and (b) its retention of BSA approximated by Thomas adsorption model.

where C_0 is the solute (BSA) concentration on the feed side (C/C_0 the penetration ratio), k_{TH} is the adsorption rate constant, M is the adsorbent mass, Q is the flow rate, C_e the maximum adsorption concentration and C_t the adsorption concentration at time t ($m_{BSA}/m_{alginate}$ in this case). Based on the correlation result (Fig. 4b), C_e was determined to be 0.205 g-BSA/g-alginate. C_e is actually the saturation point, which can be also estimated by the area under the curve of retention ratio vs. $m_{BSA}/m_{alginate}$ in Fig. 4b. BSA could not be further trapped once the gel layer was saturated.

Electrostatic interactions may play a role in this kind of adsorptive retention. Although most polysaccharides are negatively charged, many proteins posses some positively charged sites (associated particularly with the amine groups). However, given that most proteins have net negative charge, the extent of the intermolecular electrostatic interaction is unclear. Another mechanism that may contribute to adsorptive retention is associated with complexation by metal ions with metals possibly acting as bridges in protein–polysaccharide interactions.

4. Conclusions

Membrane fouling during filtration is closely related with the retention of SMP in the sludge by the membrane through formation of a gel layer. While polysaccharides, proteins and humic substances are the major components of SMP, it seems that the former two are more easily retained by the membrane. The framework of the gel layer, however, is mainly formed from polysaccharides, which controls its permeability during filtration. The SMP proteins appear to be only trapped in the gel layer rendering little structural role. Size exclusion is one possible cause for protein trapping by the gel layer. BSA retention by Ca-alginate gel layer shows that adsorptive retention may also play a role in protein trapping. The underlying mechanism for adsorptive retention needs to be investigated with particular attention given to the role of intermolecular electrostatic attraction and complexation by metal ions.

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