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Original Article

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Abstract

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The investigation of the relative roles of Soluble Microbial Products (SMP) in membrane fouling was studied in submerged membrane bioreactors (SMBRs). Three lab-scale SMBRs were operated at a constant permeate flux (12.5 l/m^2 h) with a flat sheet microfiltration membrane (hydrophilic polyolefin, pore size of 0.25 μm) using different sludge retention times (SRTs of 8, 20 and 80 days, respectively). The concentrations of SMP in terms of dissolved organic carbon (DOC) and protein at the steady-state period were compared. The results showed that as SRT increased, the organic carbon content of SMP tended to be independent of SRT while the protein content in the SMP tended to decrease with increasing SRT. Batch filtration tests were conducted to determine the specific cake resistances of the fouling layer, using both the raw sludge and the twice-washed sludge with a buffer solution. The difference in specific cake resistances in both sludges indicated that the specific cake resistance of SMP contributed approximately half the total specific cake resistance. Furthermore, SMP were found in major portions in both the biofilm and the mixed liquor. It follows that SMP played a relatively significant role in membrane fouling.

Keywords : Submerged membrane bioreactor (SMBR), Fouling, Soluble Microbial Products (SMP), Specific cake resistance.

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บทคัดย่อ

จักรกริศน์ เนื่องจำนงค์

การตรวจสอบบทบาทของผลิตภัณฑ์จากเชื้อจุลินทรีย์ที่ละลายน้ำได้ ในการเกิดฟาวลิงของเยื่อแผ่น

การตรวจสอบบทบาทของผลิตภัณฑ์จากเชื้อจุลินทรีย์ที่ละลายน้ำได้ (SMP) ในการเกิด ฟาวลิงของเยื่อแผ่น โดยศึกษาในถังปฏิกรณ์ชีวภาพจมน้ำที่ใช้เยื่อแผ่นสังเคราะห์ จำนวน 3 ถัง ดำเนินการภายใต้สภาวะที่มีระดับฟลักซ์ของเพอร์มิเอทคงที่ประมาณ 12.5 ลิตร/ตารางเมตร.ชั่วโมง เยื่อแผ่นสังเคราะห์ที่ใช้เป็นแบบแผ่น ซึ่งทำมาจากโพลีโอลีฟิน ชนิดที่มีคุณสมบัติชอบน้ำ ขนาดของช่องว่างภายในเยื่อแผ่นสังเคราะห์เท่ากับ 0.25 ไมโครเมตร โดยมีระยะเวลากักของแข็งแตกต่างกันที่ 8 วัน 20 วัน และ 80 วัน ตามลำดับ เปรียบเทียบค่าความเข้มข้นของ SMP ในรูปของคาร์บอนอินทรีย์ที่ละลายได้ (DOC) และโปรตีน ณ ช่วงเวลาที่ถังปฏิกรณ์เกิดปฏิกิริยาคงที่ในแต่ละถัง พบว่าแนวโน้มของความเข้มข้นของคาร์บอนอินทรีย์ใน SMP ไม่ขึ้นอยู่กับระยะเวลากักของแข็ง แต่ส่วนประกอบของโปรตีนใน SMP มีแนวโน้มลดลงเมื่อระยะเวลากักของแข็งเพิ่มขึ้น ทำการหาค่าความต้านทานของเค้กจำเพาะ (Specific cake resistance) ของชั้นฟาวลิง ด้วยอุปกรณ์ทดสอบการกรองขนาดเล็กที่ใช้เยื่อแผ่นสังเคราะห์ชนิดเดียวกับที่ใช้ในถังปฏิกรณ์ ทั้งจากของเหลวตะกอนในถังปฏิกรณ์แต่ละถังเมื่อเกิดปฏิกิริยาคงที่ และจากของเหลวตะกอนที่นำมาล้างด้วยสารละลายบัฟเฟอร์สองครั้ง ความแตกต่างระหว่างค่าความต้านทานทั้งสองนี้ บ่งชี้ให้เห็นถึงความต้านทานของ SMP ซึ่งมีค่าประมาณครึ่งหนึ่งของค่าความต้านทานทั้งหมด นอกจากนี้พบว่า SMP เป็นส่วนประกอบหลักของทั้งในแผ่นฟิล์มชีวภาพและในของเหลวตะกอน ดังนั้น SMP จึงมีบทบาทที่สำคัญต่อการเกิดฟาวลิงของเยื่อแผ่นสังเคราะห์

คำสำคัญ : ถังปฏิกรณ์ชีวภาพจมน้ำที่ใช้เยื่อแผ่นสังเคราะห์ ฟาวลิง ผลิตภัณฑ์จากเชื้อจุลินทรีย์ที่ละลายน้ำได้ ความต้านทานของเค้กจำเพาะ

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Introduction

Membrane bioreactors (MBRs) have been used for treating wastewater for a few decades and have been applied to treat livestock wastewater (Kim et al., 2004; Kim et al., 2004). The membrane device acts as a screen for the interception of suspended solids and micro-organisms. The accumulated soluble organic substances and specifically their effect on microbial activity has been a controversial issue. These accumulated microbial products might be one of the factors limiting bacterial activity and viability (Zhang and Yamamoto, 1996). Furthermore, the soluble organic substances that accumulate in the bioreactor were inhibitory to the metabolic activity of the activated sludge and had a negative influence on the membrane permeability to the mixed liquor (Huang et al., 2000), leading to membrane fouling. Most previous studies observed the effects of bound (extractable), extracellular polymeric substances (EPS) on membrane fouling. There is only a rare report of the effects of Soluble Microbial Products (SMP) on membrane fouling.

SMP are defined as soluble cellular components that are released during cell lysis, diffuse through the cell membrane, are lost during synthesis, or are excreted. SMP can be subdivided into two categories: substrate-utilization-associated products (UAP), which are produced directly during substrate metabolism, and biomass-associated products (BAP), which are formed from biomass, as part of decay (Laspidou and Rittmann, 2002).

This study aims to elucidate the roles of SMP in membrane fouling in SMBRs, at different sludge retention times (SRTs) during a steady-state period. The determination of SMP yield in terms of dissolved organic carbon (DOC) and protein concentration was

correlated with the degree of membrane fouling, expressed as specific cake resistances.

Material and Methods

The structure of a submerged membrane bioreactor

The laboratory scale SMBR system consisted of a flat sheet membrane (pore size of 0.25 μm), which was placed in the center of the bioreactor with a working volume of 6.25 l, as shown in Fig. 1.

The membrane was made from polyolefin, with a hydrophilic coating. The filtration area of each side of the flat membrane was 0.05 m^2, creating a total filtration area of 0.1 m^2. Three SMBRs were operated using the same hydraulic retention time (HRT) of 4.5-5.0 hours, but different SRTs, i.e. 8, 20 and 80 days. A pressure transducer was installed to monitor variations in the transmembrane pressure (TMP) between the membrane and a suction pump. A level sensor was employed in the SMBR system to maintain a constant flux at 12.5 $l/m^2.h$ The permeate was withdrawn by a suction pump under an intermittent operation mode, in a 12 min. cycle; 10 min. on and 2 min. off. The operating conditions of the SMBR system are summarized in Table 1.

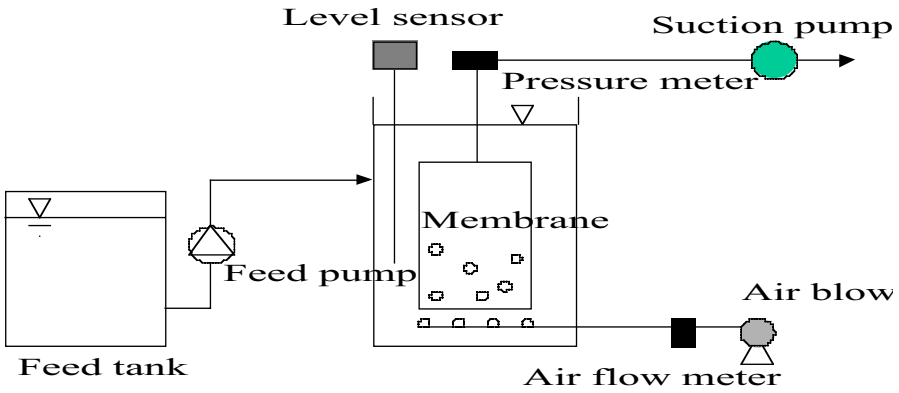

Figure 1 Schematic diagram of each SMBR.

Table 1 The operating conditions of the SMBR system.

Parameters	Operating conditions
SRT (d)	8, 20, 80
Membrane area (m^2)	0.1
Permeate flux ($l/m^2.h$)	12.5
Membrane pore size (μm)	0.25
Working volume (l)	6.25
Hydraulic retention time (h)	4.5-5.0
Air flow rate (l/min)	10
F/M ratio (kg COD/kg MLSS/d)	0.04-0.1
Operational mode	10 min of suction and 2 min of resting
Feed	Synthetic wastewater

The return sludge from the conventional activated sludge process in the Gwang-ju municipal wastewater treatment plant (Seoul, Korea) was employed to inoculate the SMBRs. The acclimatization period for the sludge to the synthetic wastewater was approximately 40 days. The synthetic wastewater was used to isolate the effects of SMP from raw wastewater influent. Glucose was used as a carbon source because it is an easily biodegradable substrate. The SMP in the bioreactors were assumed to come from microbial activity. The composition of the synthetic wastewater is presented in Table 2.

Separation of SMP

One hundred milliliter of the activated sludge was collected from each SMBR and allowed to settle for a few hours at 4°C. The supernatant was decanted. The settled sludge was then centrifuged at 12,000 g at 4°C for 15 min. The supernatant from the centrifugation was designated as SMP of the sludge flocs.

Analytical methods

The concentrations of SMP were quantified using a total organic carbon analyzer (TOC-V-CPN, Shimadzu, Japan), after the samples were passed through a 0.45 μm polypropylene membrane. Protein concentrations of SMP were quantified using a MicroBCA kit (Pierce, Rockford, USA), which is a modification of the standard Lowry method. For the protein measurement, bovine serum albumin (BSA) was used as a standard (Smith et al., 1985)

Particle size distribution of the biofilm and the mixed liquor of the SMBR was measured with three different membrane pore cut-offs (i.e. 0.1, 0.2, and 0.45 μm) after rinsing with tap water. The membranes were made from polyethersulfone (Akzo Nobel, Germany). The biofilm was collected from the fouled membrane of the SMBR with a SRT of 20 days and mixed with deionized (DI) water to create a volume of one liter. The biofilm solution was filtered through three membranes, in parallel, until 120 ml of permeate was collected. The concentration of TOC in the permeate was measured. Other analyses including the suspended solids and SCOD were performed using Standard Methods (American Public Health Association, 1998)

Table 2 The composition of synthetic wastewater.

Components	Concentration (g/l)
Phosphate buffer solution (pH 7.0):	
- KH_2PO_4	8.5
- K_2HPO_4	21.75
- $Na_2HPO_4.12\ H_2O$	44.61
$MnSO_4.7H_2O$	9.37
$FeCl_3.6H_2O$	0.25
$CaCl_2$	23.26
$MgSO_4.7H_2O$	22.5
$NaHCO_3$	0.67
$(NH_4)_2SO_4$	0.26
Glucose ($C_6H_{12}O_6$)	0.3 (SCOD 300 mg/l)

Note: The synthetic wastewater was adjusted pH = 7.2 using NaOH or H_2SO_4.

Batch stirred cell filtration test

The specific cake resistance of the cake on the membrane surface was measured using a batch stirred, cell filtration apparatus (Amicon 8200, Millipore, U.S.A.). The stirred cell unit was operated in dead-end filtration mode. A polyolefin (hydrophilic coating, pore size of 0.25 μm) membrane with a surface area of 28.27 cm^2 was used in the filtration tests. The membrane was made of the same material as was used in the SMBRs to eliminate any possible discrepancies caused by different membrane materials. The TMP and the stirring speed were regulated at 0.5 psi and 50 rpm respectively. The permeate flux was weighed with an electronic balance and the TMP values were automatically recorded using a computer programmed by Labview 5.1.

The filtration test was performed in two steps. First, DI water was used to determine the pure water flux, which was in the range of 280-360 l/m^2.h Next, the stirred cell was filled with activated sludge suspensions and allowed to filter until the volume concentration factor reached 3 (initial volume of 180 ml/ retentate volume of 60 ml).

After the batch stirred cell filtration, the flux results were used to evaluate the specific cake resistance, using the resistance-in-series model. A plot was obtained with ordinates of time/permeate volume and the abscissa of permeate volume. The slope from the plot was used to calculate the specific cake resistance (Bouhabila et al., 1998).

The specific cake resistance of the raw sludge was measured using the activated sludge in suspension. Centrifugation at 12,000 g and twice washing with buffer solution (2 mM Na_3PO_4, 4 mM NaH_2PO_4, 9 mM NaCl and 1 mM KCl at pH 7) was performed prior to determining the specific cake resistance of the bound EPS. The difference between these two specific cake resistances was assumed to be the specific cake resistance of the SMP.

Results

Overall performance of the SMBRs

The SMBRs were quite unstable during the start-up period, resulting in substantial fluctuations of TMP and MLSS. However, the SMBRs reached a steady state after approximately 60 days and relatively constant values of MLSS were observed. The overall performance in terms of water quality of the SMBRs is presented in Table 3.

Table 3 Effluent quality, removal efficiency, MLSS concentration of the SMBRs.

	COD_{cr} (mg O_2/l)	TOC (mg C/l)	MLSS (g/l)
Influent	300	115	-
SMBR1 (SRT = 8 d.)	8.0 (97.5%)	3.6 (97.2%)	3.0
SMBR2 (SRT= 20 d.)	9.0 (96.7%)	4.2 (96.4%)	4.2
SMBR3 (SRT= 80 d.)	10.0 (96 %)	3.1 (97.9%)	6.9

Investigation of TMP changes

Membrane fouling was observed by TMP changes since the SMBRs were operated at a constant flux (12.5 l/m^2.h) as shown in Fig. 2.

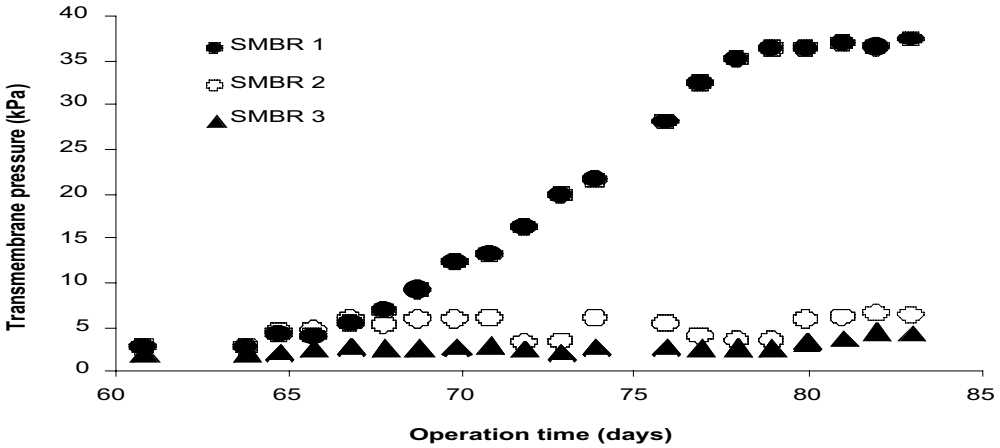

Figure 2 TMP changes during the steady-state period.

The concentrations of SMP yield

The concentrations of SMP yield were expressed in terms of DOC and protein, as shown in Fig. 3.

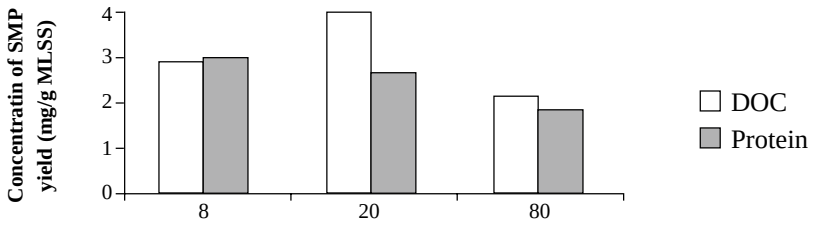

Figure 3 The DOC and protein concentrations of SMP in sludge flocs at different SRTs.

The specific cake resistances

The specific cake resistances were measured to investigate the contribution of SMP of sludge flocs to the degree of membrane fouling. The average specific cake resistance of the raw sludge (containing both the SMP of sludge flocs and bound EPS) and the twice-washed sludge (without the SMP of sludge flocs) are illustrated in Fig. 4.

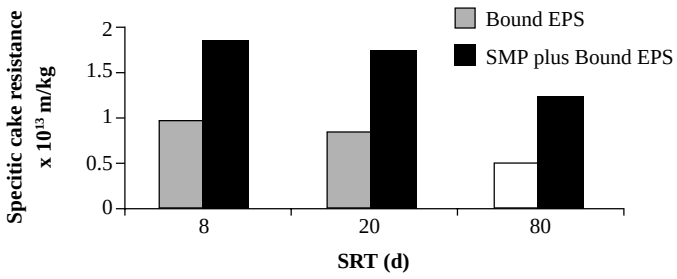

Figure 4 The specific cake resistances of raw sludge and twice-washed sludge at different SRTs.

Soluble Microbial Products (SMP)

The soluble portion of biofilm and mixed liquor in the second SMBR was observed on the twentieth day of the operation (Table 4) using the particle size distribution of the three membrane pore cut-offs.

Table 4 Particle size distribution using membrane pore cut-offs of biofilm and mixed liquor.

Pore size (μm)	TOC Weight (%)	
	Biofilm	**Mixed liquor**
< 0.1	42.68	55.26
0.1-0.2	20.65	0.58
0.2-0.45	6.55	2.87
> 0.45	30.12	41.29

Discussion

The chemical oxygen demand (COD) and total organic carbon (TOC) concentration was monitored for the removal efficiency of organic matter. The absolute retention of the particles inside the MBR by microfiltration, yielded the perfect barrier to particle matter, leading to non-detectable suspended solids in effluent. The removal efficiencies of organic matter were more than 96% for COD and 96.4% for TOC. The removal efficiency of TOC is almost identical to that of COD, because glucose is the only carbon source in the influent.

The SMBRs were operated using a constant flux mode in this study. TMP was monitored to investigate membrane fouling. TMP was stable over the 60-67 day period in all three SMBRs. After the 67th day, three SMBRs started to show differences in TMPs. The SMBR with a SRT of 8 days had a substantial increase in TMP while the SMBRs with SRTs of 20 and 80 days had stable TMPs. Although the difference was minor, TMPs of the SMBR with a SRT of 80 days remained higher than those with a SRT of 20 days, until the end of the operation. The results indicated that membrane fouling decreased with increasing SRTs. Similar results have been reported by research under different operation conditions (Chang and Lee, 1998).

In this study, the amount of SMP was quantified during the steady-state period. The average concentrations of SMP were 2.93 (±0.71), 3.77 (±2.8) and 2.2 (±1.74) mg DOC/g MLSS for SRTs of 8, 20 and 80 days, respectively. However, the concentrations of DOC sludge flocs were independent of SRTs. The average protein concentrations of SMP were 3.07 (± 2.41), 2.6 (± 1.26) and 1.62 (± 0.89) mg BSA/g MLSS for SRTs of 8, 20, 80 days, respectively. The protein concentrations tended to decline with increasing SRTs.

In addition to bound EPS (not shown in this paper), a high concentration of SMP protein at the SRT of 8 days is probably related to the rapid TMP increase in the SMBR 1. It could be because protein was thought to play a role in the adhesion process in the initial biofilm formation (Danielsson et al., 1977 cited by Jahn and Nielsen, 1995). Moreover, the SMP of sludge flocs also played a relatively vital role in membrane fouling, because the substrate for the SMBRs was highly biodegradable compounds (i.e., glucose), and the DOC removal was more than 96 percent.

To investigate the degree of fouling, the specific cake resistances were determined in batch stirred, cell filtration tests. The average specific cake resistances of the twice-washed sludge were 9.61 (± 3.35) x 10^{12}, 8.78 (± 7.23) x 10^{12}, and 4.51 (± 2.57) x 10^{12} m/kg for SRTs of 8, 20 and 80 days, respectively. Those of the raw sludge were 1.83 (± 2.1) x 10^{13}, 1.77 (± 1.64) x 10^{13} and 1.26 (± 1.24) (x10^{13} m/kg, respectively. The results showed that the specific cake resistances of both sludges tended to decrease with increasing SRTs. The potential for membrane fouling was therefore less with prolonged SRTs.

It was noted that the difference in the specific cake resistances between both sludges showed that approximately 50 % of the total specific cake resistance of the raw sludge was mainly from the SMP of sludge flocs.

The soluble portions could be considered as SMP and act as a major foulant. Generally, the total solid content is divided into suspended (> 0.45 μm) and soluble (< 0.45 μm) matter. The results (Table 4) showed that the soluble portions of biofilm and mixed liquor were 70% and 59% respectively. Furthermore, the smallest particle size (<0.1 μm) was the major component of both biofilm and mixed liquor. Huang et al. (2000) reported that the smallest organic substance in SMP (in terms of the supernatant TOC) was mainly found in SMBR after long-term operation.

However, in the case of SMBR applied to livestock wastewater treatment, the role of SMP in membrane fouling may be different from this work. It is therefore significant to pursue research into the relationship between the SMP concentration and the degree of membrane fouling.

Conclusions

SMP of sludge flocs played a relatively significant role in membrane fouling though their DOC concentrations were independent of SRTs. Protein concentrations in SMP tended to relate to the degree of fouling at different SRTs. The specific cake resistance of SMP contributed to approximately 50 % of the total specific cake resistance of the raw sludge. Moreover, biofilm and mixed liquor were largely composed of soluble portions. It is concluded that SMP could be an important cause of membrane fouling.

Acknowledgments

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