Dissertation Abstract

Effects of Microbial Quorum Sensing on Membrane Fouling and Characteristics of Extracellular Polymeric Substances in a Membrane Bioreactor Process

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Abstract

Membrane fouling is deposition of extracellular polymeric substances (EPS) on membrane surface or within pores, as well as biofilm formation via microbes on membrane surface, which is the most challenge of membrane bioreactor (MBR) process. EPS characteristics and bacterial structure are considered as vital factors causing membrane fouling, which is positively related to microbial quorum sensing (QS) process. Lab-scale MBRs of microfiltration process were operated to investigate key EPS components and microbes causing fouling, and to control fouling via QS inhibition (vanillin addition). Hydrophobic EPS lower 100 kDa are adsorbed into membrane at initial stage of fouling. Hydrophilic EPS of 100-670 kDa highly attached onto membrane pores and caused irreversible fouling. Hydrophilic EPS larger than 670 kDa deposited on membrane surface to form cake layer. QS bacteria, EPS producers and EPS hydrolyzers (Comamonadaceae, Xanthomonadaceae, and Chitinophagaceae) were dominant in bulk sludge but only Xanthomonadaceae were highly abundant in cake layer (or biofilm). QS inhibition via vanillin was able to reduce fouling by reducing Xanthomonadaceae and Chitinophagaceae, reducing productions and hydrophobicity of EPS in bulk sludge and cake layer. Vanillin inhibited QS activity and increased abundance of Flavobacterium (AHL producers) but did not inhibit bacterial growth and AHL production.

1. Introduction

Membrane fouling is the most challenge of membrane bioreactor (MBR) process because it causes poorer membrane permeability, serious flux decline; resulting in higher costs for more energy consumption, more frequent membrane cleaning and replacement (Deng *et al.*, 2016). Fouling is defined as deposition of suspended or dissolved substances on membrane surface or within membrane pores (Koros *et al.*, 1998), causing ether a decline of permeate flux or an increase of transmembrane pressure (TMP) (Le-Clech *et al.*, 2006). Fouling mechanisms include (i) pore narrowing/ clogging; (ii) gel layer and cake layer formation; and (iii) biofilm formation – growth of microbes on membrane surface (Wu *et al.*, 2012; Lin *et al.*, 2014). Sludge flocs, cells, particles, colloids, extracellular polymeric substances (EPS) can deposit to membrane causing fouling (Lin *et al.*, 2014), in which EPS are considered as primary foulants.

Extracellular polymeric substances are macromolecular polymers secreted from microorganism, which also contain organic matters from wastewater and products of macromolecular hydrolysis (Sheng *et al.*, 2010). Polysaccharides, proteins are primary components of EPS (Frolund *et al.*, 1996). Nucleic acids, lipids and other compounds are also present at low contents. EPS are often fractionated into soluble microbial products (SMP), loosely-bound EPS (LB-EPS), and tightly-bound EPS (TB-EPS) (Nielsen and Jahn, 1999; Pellicer-Nàcher *et al.*, 2013). SMP can be released from bound EPS and vice versa bound EPS also adsorb SMP into their matrix (Ni *et al.*, 2011; Shi *et al.*, 2017). EPS in MBRs determine sludge properties including charge, hydrophilicity/ hydrophobicity, molecular weight (MW) distribution, which are important for flocculation, deposition of foulants causing membrane fouling (Lin *et al.*, 2014; Nguyen *et al.*, 2014). For these reasons, EPS content and characteristics have been expected to determine the extent and severity of fouling condition (Silva *et al.*, 2016). However, membrane fouling caused by EPS is not comprehensively understood and still controversial among researches.

Bacterial community and EPS production are believed to be important factors causing membrane fouling, which are reportedly affected by operational conditions (Cho *et al.*, 2006; Guo *et al.*, 2015; Deng *et al.*, 2016). Especially, biofilm formation of microbes on membrane surface causes TMP jump and increases fouling severity. Investigations about microbial community in MBRs have been conducted (Ma *et al.*, 2013; Guo *et al.*, 2015, Jo *et al.*, 2016). Nonetheless, findings and knowledge about microbial ecology of cake layer are limited to understand and control membrane fouling. For this reason, bacterial structure of cake layer (known as biofilm) of MBRs under different filtration conditions is still one of the hottest topics in membrane fouling study.

Recently, EPS production and biofilm formation causing membrane fouling have been found to have positive relation to microbial quorum sensing (QS) process (Yeon *et al.*, 2009; Lade *et al.*, 2014; Si and Quan, 2017). In that process, microbes produce and sense chemical signals (e.g., Acyl homoserine lactones - AHLs) of cell population density to regulate collective behavior including biofilm formation (Waters and Bassler, 2005). Inhibition of microbial QS process is believed to reduce biofilm formation on membrane surface, resulting in a mitigation of membrane fouling (Siddiqui *et al.*, 2015). Investigation about QS inhibition via bacteria, enzyme and natural compounds (e.g., vanillin) to control membrane fouling control have just been started (Oh *et al.*, 2012; Lee *et al.*, 2016). It requires comprehensively experimental knowledge about possible effects of QS inhibition on EPS characteristics, bacterial community and membrane fouling.

This study aimed to understand EPS characteristics in MBRs and to control membrane fouling via quorum sensing inhibition. Lab-scale MBRs were operated to investigate key EPS components, microbes causing membrane fouling and effects of vanillin on regulation of QS, membrane fouling.

2. Materials and Methodology

2.1. EPS extraction method

Several extraction methods have been applied to extract EPS; however, extraction methods possibly affect EPS components. Hence, choosing appropriate EPS extraction methods is important before making investigations about EPS characteristics causing membrane fouling. Three efficient methods (HCHO/NaOH, NaOH/Heat, cation exchange resin – CER) were compared in extraction efficiency, cell lysis and EPS components to choose a suitable method for TB-EPS extraction.

2.2. Lab-scale MBR operation

To investigate key EPS components causing membrane fouling, a lab-scale MBR was operated under aerobic condition to treat artificial sewage. Operational condition and ingredients of artificial sewage were described in Table 2.1. This study employed microfiltration flat sheet membrane (average pore size at 0.2 μm, chlorinated polyethylene, Kubota, Japan).

Table 2.1. MBR operational condition and ingredients of artificial sewage

Operational parameters		Artificial sewage	[mg/L]
Working volume	2 L	Glucose	250
Membrane area	152 cm^2	Peptone	40
MLSS	5 - 7 g/L	Yeast extract	40
Filtration/relaxation	4 min/ 1 min	$NaHCO_3$	190
HRT, SRT	12 – 14 h, 28 days	KH_2PO_4	10
Physical cleaning	brushing (weekly)	$CaCl_2$	99
Temperature	18 – 22 ^oC	$FeCl_3 \cdot 6H_2O$	2
Organic loading rate	40–60 mg TOC/g·day	NH_4Cl	80
TOC influent	120-160 mg/L	$MgSO_4 \cdot 7H_2O$	80
TOC removal	93-98 %	$Mg(NO_3)_2 \cdot 6H_2O$	3

To study EPS production and bacterial community under different filtration conditions, a lab-scale MBR was operated under two stages: Stage 1 was a operation of constant flux at 9-11 $L/m^2/h$ for three months, followed by Stage 2 of a constant TMP operation of 80 kPa for next three

months (Table 2.2). Other operational parameters were operated as in Table 2.1.

Table 2.2. MBR operation for investigation of EPS production and bacterial community

Operational condition	Stage 1 - constant flux	Stage 2 - constant TMP
Flux	9-11 LMH	-
TMP	-	80 kPa
Permeability	0.27 ± 0.08 LMH/kPa	0.18 ± 0.02 LMH/kPa

For investigation about effect of vanillin on membrane fouling, 2 lab-scale MBRs were operated to treat artificial sewage. One MBR was continuously feed with artificial sewage containing vanillin at a dose of 250 m/L (MBR-vanillin) and the other was feed only with artificial sewage as a control MBR (MBR-control). Working volume, HRT, and SRT were at 1.6 L, 7-8 h, and 25 days, respectively.

2.3. EPS extraction and characterization

Fouled membrane was taken out of aeration tank for physical cleaning by brushing. Collected foulant on membrane surface was considered as cake layer sample. EPS extraction method for this study were chosen after evaluating effects of methods on extraction efficiency, cell lysis and EPS components. EPS from bulk sludge and cake layer were fractionated into SMP, LB- and TB-EPS via centrifuge (6000 ×g, 10 min), ultra-sonication (3.5 W/mL, 2 min) and HCHO/NaOH (6 μL/mL; 0.4 mL/mL). Protein, polysaccharide components and EPS content were quantified in modified Lowry method, phenol-sulfuric acid method and TOC analyzer, respectively. EEM spectra, polarity and MW of EPS were characterized via a fluorescent spectrophotometer (FP-8200, Jasco), a HPLC-ELSD detector, equipped with a SEC column (OHpak SB–806M, Shodex) and a reversed-phase column (C18M 4D, Shodex).

2.4. Bacterial community analysis

DNA extract from environmental samples (e.g., bulk sludge, cake layer) was used as templates

in the 1st-PCR to amplify V3-V4 regions of 16S rRNA genes, in which a primer pair for the PCR was appended with overhang adaptors. The 2nd-PCR was conducted to amplify 1st-PCR products and add sequencing adaptors. To sequence many samples at once, barcodes or indices (unique sequences of 10-12 bp) were also appended into the overhang and sequencing adaptors. These barcodes (or indices) were used for sample identification in downstream analysis. The 2nd-PCR amplicon was quantified and pooled prior to be sequenced via Illumina Miseq platform based on paired-end reading method.

To analyze bacterial community, raw sequence data were trimmed to remove fragments with low-quality, short sequences. The trimmed reads were merged to make paired-end library. Subsequently, the paired-end library was remove chimeric sequences via UCHIME function of USEARCH program prior to be used for bacterial community analysis via –QIIME (v1.9.1), in which Greengenes database of 97% similarity were used.

3. Results and Discussion

3.1. Optimum methods for EPS extraction

Among the three methods, NaOH/Heat obtained the highest efficiency but could cause cell lysis, which resulted in overestimation. HCHO/NaOH had higher extraction efficiency and less cell lysis than CER. Moreover, there were no remarkable differences in protein and polysaccharide contents among extraction methods. However, they affected molecular weight and fluorescent organic components. NaOH/Heat caused hydrolysis EPS in extract while CER could not extract large MW components of EPS. HCHO/NaOH was chosen for TB-EPS extraction because this method did not cause these effects as NaOH/Heat and CER.

3.2. Key EPS components causing membrane fouling

Hydrophobic substances smaller than 20 kDa were mainly found in SMP of bulk sludge (Figure 3.1). SMP were highly adsorbed into membrane at initial stage of filtration. Hydrophobic fraction of SMP, which contained a high content of humic acid-like substances, was possibly released from hydrophobic fraction of LB-EPS in bulk sludge. It was because these fractions shared similarities in EEM spectra and polarity-MW profile (Figure 3.1).

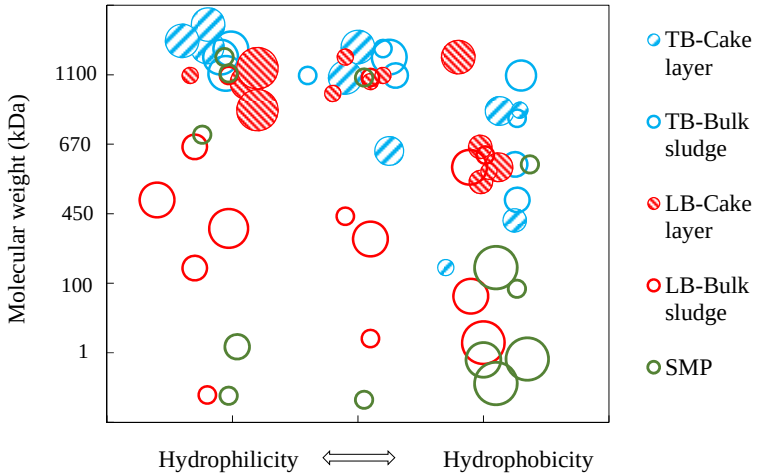

Figure 3.1. Polarity – MW profiles of EPS fractions in bulk sludge and cake layer (n=4).

Hydrophilic substances larger than 450 kDa were found in TB-EPS of bulk sludge, LB- and TB-EPS of cake layer (Figure 3.1). EEM spectra, polarity – MW profile of EPS fractions in cake layer were highly similar with those of TB-EPS in bulk sludge. Hence, TB-EPS of bulk sludge probably deposited on membrane surface as a main component of EPS in cake layer.

Besides containing hydrophobic substances, LB-EPS of bulk sludge were also comprised of hydrophilic substances 100-670 kDa (Figure 3.1), which were not found in cake layer, permeate nor effluent of membrane backwash. These phenomena revealed that these substances could not

be removed by cleaning, possibly causing irreversible fouling.

3.3. Bacterial community under different filtration conditions

Comamonadaceae, *Xanthomonadaceae* and *Chitinophagaceae* were the most dominant families in bulk sludge while only *Xanthomonadaceae* were highly dominant in cake layer. Different filtration conditions had effects on bacterial community, causing a shift of in bacterial structure of constant TMP (Stage 2) from constant flux (Stage 1). The constant TMP enhanced differences in the structure between cake layer and bulk sludge in Stage 2 (Figure 3.2). Abundance of OTUs which phylogenetically closed to biofilm and QS bacteria, had positive correlation to SMP and EPS content.

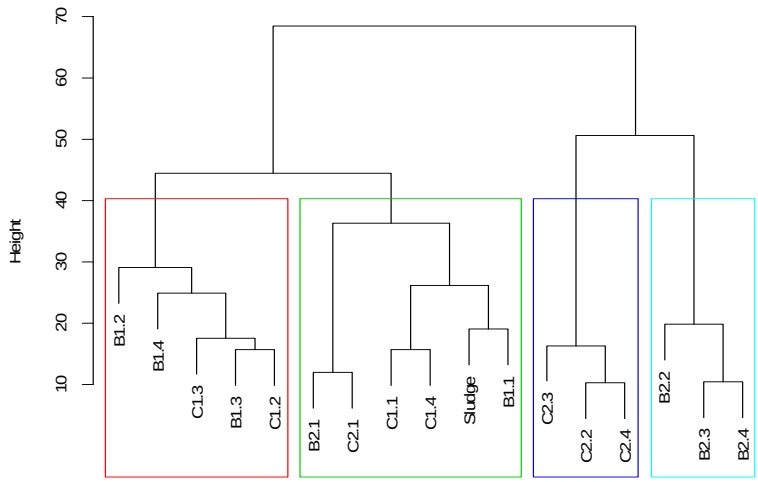

Figure 3.2. Clustering analysis based on bacterial communities at genus level under two stages. (B1.1-4 and C1.1-4: communities of bulk sludge and cake layer in Stage 1; B2.1-4 and C2.1-4: communities of bulk sludge and cake layer in Stage 2).

3.4. Effect of vanillin addition on MBR performance

Vanillin addition into MBRs was expected to inhibit microbial QS process regulating biofilm formation on membrane surface, further to reduce membrane fouling (Nam *et al.*, 2015). In this study, vanillin had certain effects on QS process, bacterial community, EPS production and characteristics in bulk sludge and cake layer, which reduced TMP development (Figure 3.3).

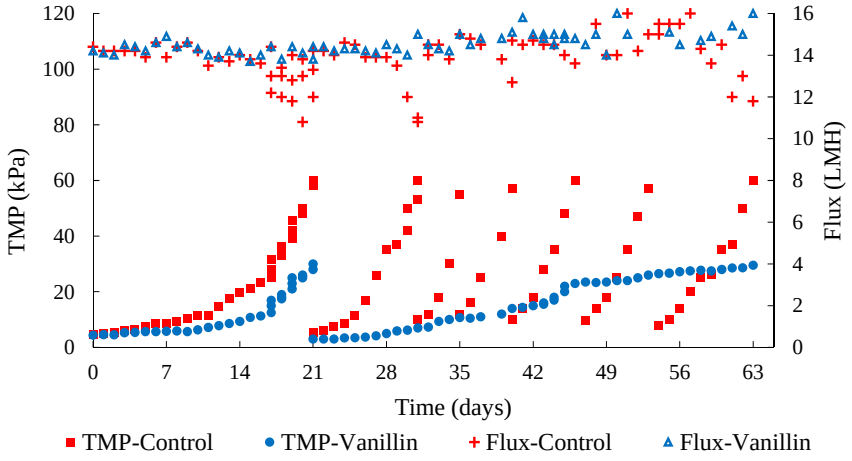

Figure 3.3. Fouling reduction (reduction of TMP development) via vanillin.

In bulk sludge, presence of vanillin led to an increase of relative abundance of *Flavobacterium* QS bacteria (Wagner-Döbler *et al.*, 2005) but decreases of *Xanthomonadaceae* and *Chitinophagaceae* - producer and hydrolyzer (Szabó *et al.*, 2017) (Figure 3.4). Vanillin also caused reductions of EPS production (especially, hydrophobic EPS) in LB-EPS and TB-EPS (Figure 3.5), resulting in a lower content of hydrophobic substances in SMP and higher flocculation of bulk sludge. This phenomena possibly lessened deposition of EPS on membrane surface, which was important for initial fouling stage.

On membrane surface, vanillin possibly inhibited QS process of abundant microbes, which involved QS bacteria (e.g., *Pedobacter, Flavobacterium*) (Lv *et al.*, 2014), and EPS producers (*Xanthomonadaceae* bacterium) (Szabó *et al.*, 2017) (Figure 3.4). Hence, vanillin might cause a reduction in EPS production from microbes on membrane surface (Figure 3.6), contributing to 36 % reduction of cake layer formation. In addition, vanillin decreased content of hydrophobic fraction in SMP, LB- and TB-EPS of cake layer, which possibly offered advantages for the reductions of TMP development and membrane fouling (Figure 3.3).

Class_Family_Genus	Bulk sludge		Cake layer	
	Control	Vanillin	Control	Vanillin
*Saprospirae_Chitinophagaceae*_Unclassified genus	29.2	0.8	25.4	2.4
*Gammaproteobacteria_Xanthomonadaceae*_Unclassified genus	22.1	11.7	3.9	1.7
*Betaproteobacteria_Comamonadaceae*_ Unclassified genus	15.0	7.8	3.5	1.6
Gammaproteobacteria_Xanthomonadaceae_Lysobacter	5.8	0.0	2.4	0.0
*Betaproteobacteria_Comamonadaceae*_ Unclassified genus	1.8	13.5	0.5	6.9
Flavobacteriia_Cryomorphaceae_Fluviicola	0.3	0.8	2.5	27.4
*Cytophagia_Cytophagaceae*_Unclassified genus	0.2	0.0	20.4	0.3
Flavobacteriia_Flavobacteriaceae_Flavobacterium	0.2	43.0	0.0	7.9
TM7-3_Unclassified family_Unclassified genus	0.1	1.1	2.6	9.9
TM7-3_Rs-045_Unclassified genus	0.1	0.0	11.9	0.0
Verrucomicrobiae_Verrucomicrobiaceae_Prosthecobacter	0.0	1.1	0.0	15.4
Sphingobacteriia_Sphingobacteriaceae_Pedobacter	0.0	0.0	0.0	13.4

Figure 3.4. Effect of vanillin addition on bacterial community (relative abundance of the most dominant genera).

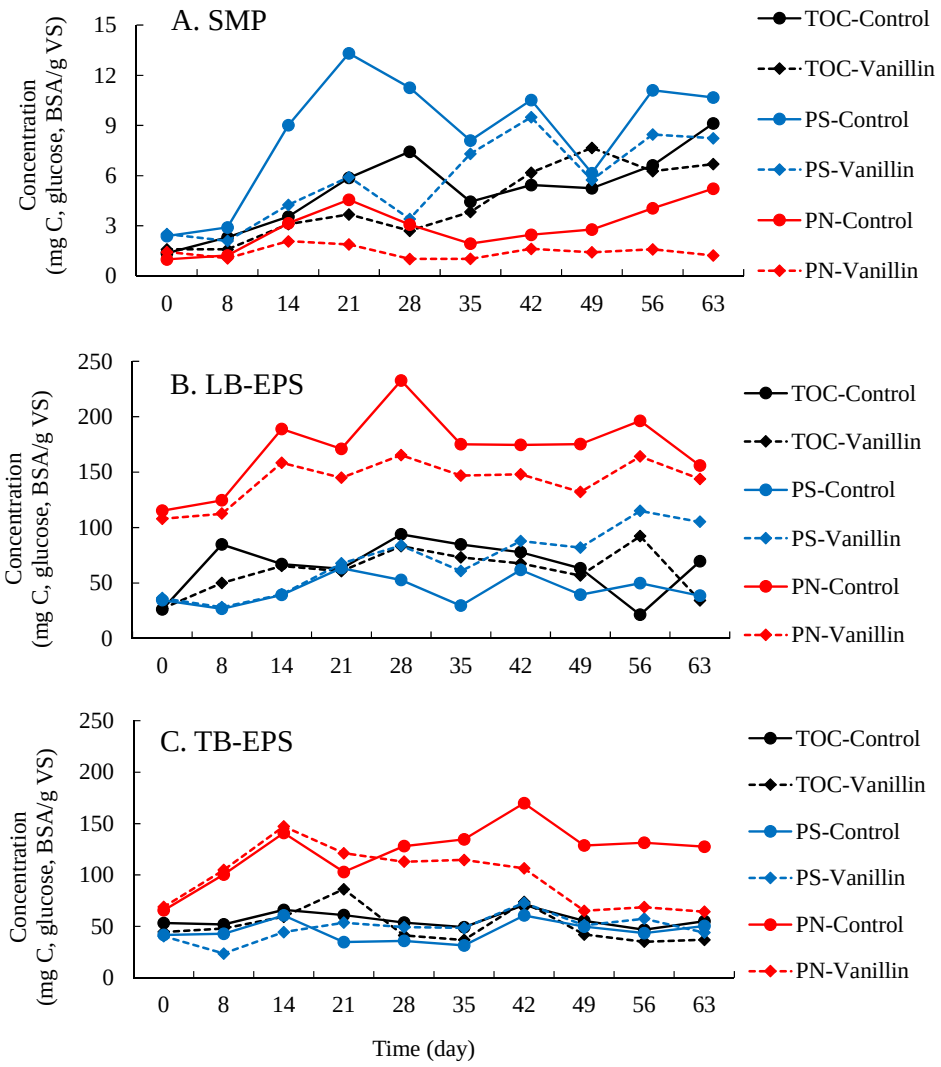

Figure 3.5. EPS content in bulk sludge under vanillin addition

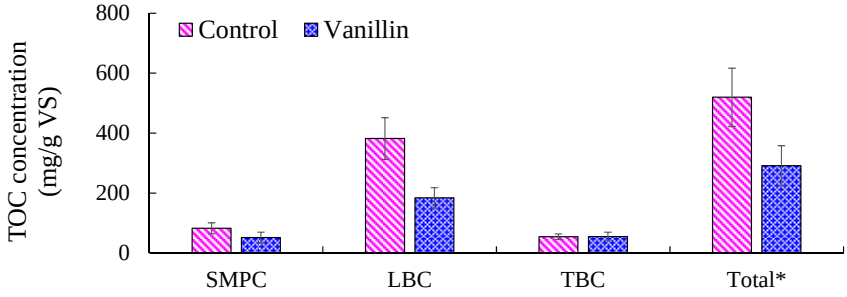

Figure 3.6. EPS content in cake layer under vanillin addition (n=2)

4. Conclusions

Each EPS fraction, polarity fraction and MW size obtained its own different fate to cause membrane fouling. Hydrophobic SMP (<100 kDa) that highly released from LB-EPS fraction condition membrane pores. Subsequently, hydrophilic biopolymers (100-670 kDa) in LB-EPS narrow conditioned pores, causing irreversible fouling. While, hydrophilic substances > 670 kDa in TB-EPS attach on conditioned surface to form cake layer.

Comamonadaceae and *Xanthomonadaceae* bacteria are highly abundant in bulk sludge and have positive correlation to SMP and EPS production while only some *Xanthomonadaceae* bacteria are greatly potential adsorption into membrane surface. Moreover, filtration conditions (e.g., constant flux, constant TMP) have different influences on bacterial structure. A constant TMP operation enhances distinctions in bacterial structure between bulk sludge and cake layer.

Quorum sensing inhibition via vanillin has effects on community structure, EPS production and characteristics in bulk sludge and cake layer, which reduced TMP development. Vanillin causes a shift in bacterial structure of bulk sludge, reducing EPS producers and hydrolyzers (e.g., *Xanthomonadaceae* and *Chitinophagaceae*). Vanillin also causes reduction of EPS production in bulk sludge and cake layer, reduction of cake layer (biofilm) formation.

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