

Soluble Microbial Products Removal Profile and Morphological Assessment of Submerged Ultrafiltration Membrane

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Abstract: Performance of ultrafiltration membranes were investigated with submerged membrane in terms of removal of soluble microbial products (SMP) (as proteins and carbohydrates) and fouling mechanisms. Cellulose (UC) and polyethersulphone (UP) membranes with different molecular weight cut off (MWCO) (5, 10, 30 kDa for UC and 5, 10, 20 kDa for UP) were tested in the bioreactor. The quality of permeate was compared in terms of SMP and COD. There was no significant difference in the total SMP removal effectiveness for both the UC and UP membranes with different MWCO characteristics. However, UP membranes were relatively more effective in removing soluble carbohydrates, while UC membranes were more effective in removing soluble proteins. The submerged membrane bioreactor achieved organic removal efficiencies ranging from $98.1 \pm 0.2\%$ to $99.2 \pm 0.3\%$ based on the soluble COD levels. Analysis of the membrane performance data by resistances-in-series model indicated that cake fouling was the dominant membrane fouling mechanisms. Increasing the MWCO was resulted in higher membrane flux but lower SMP removal. Morphological examination of the membranes by SEM and AFM showed significant accumulation of organisms on the membrane surface.

Keywords: Submerged membrane bioreactor, ultrafiltration, membrane fouling, soluble microbial products (SMP).

1. INTRODUCTION

Submerged membrane bioreactors (SMBRs) for wastewater treatment and water reuse applications have received significant interest in recent years as they offer operational and design benefits by eliminating the need for a secondary settling tank, require smaller reactor volume, and decrease sludge volume and quantity. However, limited experience available on sludge characteristics in the SMBRs and sensitivity of system performance to operating conditions restricts their wider applications. Performance characteristics of submerged membrane bioreactors with micro and macro systems have been studied to evaluate the effect of operational parameters on sludge filterability and process performance [1, 2]. Physiological properties of sludge from SMBRs have also been studied in terms of extracellular polymeric substances [3-5], carbohydrates and proteins [6], and soluble microbial products [7, 8].

Membrane fouling is one of the major operational concerns of SMBRs. It is a growing research area to understand membrane performance in relation to system parameters. Extracellular polymeric substances (EPS) or soluble microbial products (SMP) are large molecular weight compounds that are released by bacteria. They consist of proteins, polysaccharides, lipopolysaccharides, lipoproteins or complex mixtures of these biopolymers with a variety of functional sites

including carboxyl, amino and phosphate groups. In recent years, research on polymeric substances either in soluble or bound form has gained increasing interest [9]. However, the current understanding of membrane fouling mechanisms and membrane performance in relation operational parameters in SMBRs are still limited. EPS plays an integral part of the biofilm structure and development especially for attachment, detachment, mechanical strength, and protection against environmental stress factors. The bioadhesive characteristics of EPS alter the original surface properties rendering hydrophobic surfaces to become hydrophilic. Once the EPS deposit on surfaces, they can provide sources of nutrition for bacteria, and create the conditions necessary for bacterial attachment [10].

Ultrafiltration (UF) processes are able to separate the majority of particles and microorganisms from raw water, and considered as the alternative to conventional clarification and filtration units [11]. So far, numerous studies were carried out to investigate the causes, characteristics and mechanisms of MBR fouling, and to develop more efficient methods for membrane fouling mitigation [12, 13]. Different pre-treatment processes to remove relevant foulants from treated wastewater have been investigated up to the present [14-19]. However, there is still little information available with regard to the impact of membrane type and material on MBR fouling, and thus further in-depth investigation is required.

The purpose of the study was to evaluate the performance of submerged membrane modules in a

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continuous bioreactor system in relation to removal of soluble microbial products (SMP) (as proteins and carbohydrates) and membrane fouling mechanisms. Cellulose (UC) and polyethersulphone (UP) membrane modules with different molecular weight cut off (MWCO) (5, 10, 30 kDa for UC and 5, 10, 20 kDa for UP) were placed in the bioreactor operated with synthetic domestic wastewater. The quality of the effluent from the bioreactor was compared with the quality of the filtrates for SMP and COD. Membrane fouling mechanisms were analyzed by resistances in series model. Morphological assessments were performed by scanning electron microscopy (SEM) and atomic force microscopy (AFM).

2. MATERIALS AND METHODS

2.1. Submerged Membrane Bioreactor

A submerged membrane bioreactor (SMBR) with an effective volume of 50 L was used to treat the synthetic municipal wastewater [20]. The MBR system was operated with synthetic wastewater to control the operating conditions and evaluate the system performance in a systematic manner. The synthetic wastewater composition was given in our previous study [20]. All reagents were of analytical grade. The seed sludge was obtained from the sedimentation tank of Gebze Wastewater Treatment Plant in Turkey.

Influent and returned sludge were fed to the bioreactor with a peristaltic pump (Cole Parmer

Masterflex) and a diaphragm dosaging pump, respectively. Compressed air was supplied by a peripheral diffuser which was placed at the base of the bioreactor. The air supplied by the diffuser provided mixing of suspension in the bioreactor and scouring of the membrane surface. The dissolved oxygen concentration in the bioreactor was 4.6 ± 1.1 mg/L. The hydraulic retention time (HRT) was kept at 12 h, and sludge retention time (SRT) was 10 days. Excess sludge was withdrawn daily at a rate of 5 L/day from of the bioreactor to maintain the concentration of MLSS at 3394 ± 254 mg/L at 10 days. The sludge temperature was controlled at 22.4 ± 1.9 °C with an electric heater. Detailed operational conditions of the bioreactor system are summarized in Table 1. After 60 days, the bioreactor operation was terminated and the supernatant was analyzed.

The flat sheet membrane modules, made of polypropylene, had a total area of 50 cm² each. The modules were operated under constant suction pressure (-140 ± 5 mmHg) and the membrane flux was monitored by measuring the amount of filtrate produced over time. Each filtration run was conducted for 24 h.

2.2. Membrane Characteristics

Cellulose (UC) and polyethersulfone (UP) ultrafiltration membranes by Microdyne Nadir, Germany were used in this study. Three types of cellulose (UC) membranes with MWCO of 5, 10, 30 kDa and three types of polyethersulfone (UP) membranes with MWCO of 5, 10, 20 kDa were used. Three membrane

Table 1: Operating Conditions of Bioreactor and Submerged Membrane Modules

System parameters	Value	Unit
Influent		
COD	610±52	mg/L
BOD ₅	450±40	mg/L
Bioreactor		
Bioreactor volume	50	L
Sludge retention time (SRT)	10	d
Hydraulic retention time (HRT)	12	h
Hydraulic flow rate	100	L/d
F/M ratio	0.36	1/d
MLSS concentration	3394±254	mg/L
MLVSS concentration	3061±235	mg/L
Organic loading rate (OLR)	1.3	kg COD/m ³ d
Temperature	22.4±1.9	°C
Dissolved oxygen (DO)	4.6±1.1	mg/L
pH	7.3±0.2	-
Specific air demand based on membrane area (SAD _m)	0.6	m ³ air/m ² membrane area/h

modules were placed in parallel mode inside the bioreactor. The pressure inside the vacuum tank was measured with a vacuum pressure gauge (0–1 bar). The permeate quantities were measured in real time with an electronic balance (Schimadzu, Japan) and data were recorded on a RS 232 and PCI card.

2.3. Membrane Resistance Analysis

Membrane resistances were evaluated by the resistance-in-series model as follows (Eq. 1):

$$R_t = R_m + R_p + R_c = \frac{\Delta P}{\mu J} \quad (1)$$

At the end of each run, the extent of membrane fouling was quantified by measuring permeate flux at constant suction pressure (-140±5 mmHg). The membrane resistances were determined from the flux data given in our previous study [21].

2.4. Physico-Chemical Analysis

Measurements of chemical oxygen demand (COD), biochemical oxygen demand (BOD₅), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and sludge volume index (SVI) were performed according to procedures described in APHA Standard Methods [22]. Samples for soluble COD were obtained by filtration of the mixed liquor through filter paper (cellulose acetate) with mean pore size of 0.45 μm. The pH measurements were carried out with a glass electrode (WTW multi 340i model pH meter, Multi Parameter Instrument). DO concentration and temperature in the bioreactor were measured by a DO meter (HACH HQ 40d multi).

The EPS were extracted using formaldehyde–NaOH extraction method in accordance with Li's *et al.* [23]. The measurement of protein content was carried out according to Lowry methods [24]. BSA was used as a standard and the results expressed in mg equivalent of bovine serum albumin (BSA) per liter. Polysaccharides were determined the phenol-sulfuric acid method of Dubois *et al.* [25]. Glucose was used as a standard and the results expressed in mg equivalent of glucose per liter. All samples were determined the concentrations using a UV–vis spectrophotometer (GBC-Cintra-20) at the wavelength of 660 nm for protein or at the wavelength of 490 nm for polysaccharide.

Membranes were examined by a scanning electron microscope (SEM) (Philips XL30 SFE Scanning

Electron Microscopy) before and after the filtration runs. For SEM analyses, a small membrane sample (0.5 × 0.5 cm) was used. The membrane samples were fixed with 3.0% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2, dehydrated with ethanol (95%), and dried at room temperature. The samples were gold coated prior to examination by SEM.

Atomic force microscopy (AFM, Digital Instruments) was used to examine the surface morphology of the membranes. Before AFM observations, both the used and clean membranes were gently washed with deionized water and dried at room temperature. The membrane samples were fixed on glass slides and scanned over 10.0 μm × 10.0 μm. AFM was performed in tapping mode at a scanning rate of 6.104 Hz. The images were analyzed using Nanoscope 3.0 software.

4. RESULTS AND DISCUSSION

4.1. SMP and COD Removal

Effectiveness of UF membranes with different MWCO were evaluated for SMP removal as total (SMP_t), carbohydrate (SMP_c), and protein (SMP_p). The SMP levels in the filtrates were evaluated for protein and carbohydrate fractions and compared with those in the effluent from the bioreactor. Figure 1 presents a comparison of the SMP concentrations in the filtrate collected from each membrane and compares with those in the effluent from the bioreactor. The SMP_t levels in the filtrates ranged from 4.70 to 8.05 mg/L which was significantly less than the SMP_t in the bioreactor effluent (21.09 mg/L). There was no significant difference in the removal effectiveness of SMP_t for both the UC and UP membranes with different MWCO characteristics. In addition, there was no significant difference in SMP_t removal between the UP membranes and UC membranes. However, the UP membranes were relatively more effective in removing soluble carbohydrates while UC membranes were relatively more effective in removing soluble carbohydrates. The soluble carbohydrate levels in the filtrates for the UP membranes ranged from 1.12 to 1.75 mg/L and for the UC membranes they were between 3.97 and 4.02 mg/L. The soluble protein levels in the filtrates for the UC membranes ranged from 0.87 to 1.22 mg/L and for the UP membranes it ranged from 2.95 to 6.93 mg/L. Filtration performances and fouling behaviours of polyacrylonitrile (PAN) and polyvinylidene fluoride (PVDF) membranes were studied in a pilot-scale membrane bioreactor by Wang *et al.* [26]. Results showed that removable fouling was

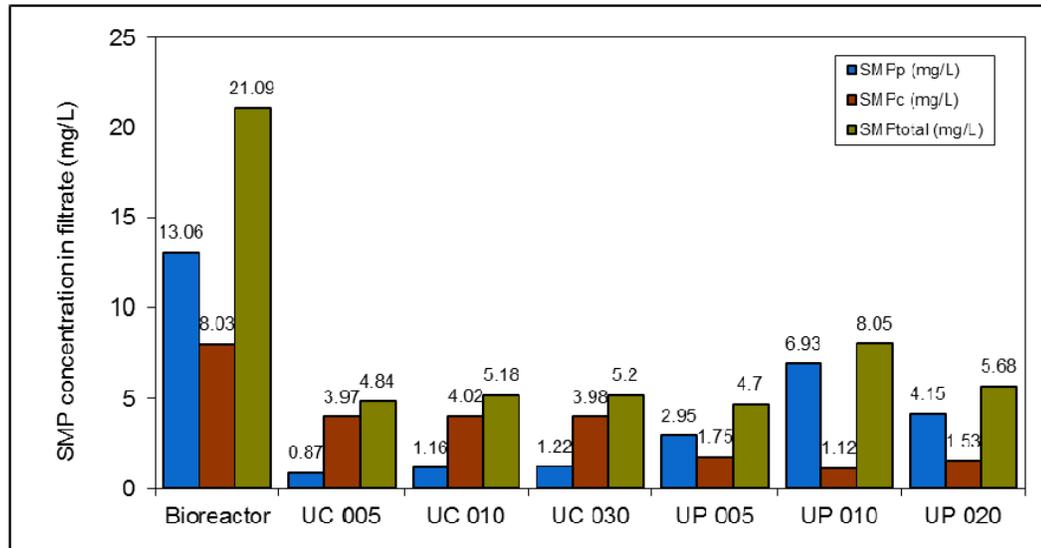


Figure 1: SMP levels in the bioreactor effluent and filtrates from SMBR.

dominant for both membranes while irremovable fouling of the PVDF membrane was severer than that of the PAN membrane. The PAN membrane could reject more soluble microbial products (SMP) than the PVDF membrane due to different pore sizes. The PVDF membrane rejected more carbohydrates and fewer proteins than the PAN membrane. The mean particle size of surface foulants on the PAN membrane was smaller than that on the PVDF membrane [26].

Influent COD to the bioreactor was 610 ± 52 mg/L, whereas effluent COD from the CASP system was about 64 ± 19 mg/L and COD levels of the permeates from the SMBR were less than 11 mg/L for all the membranes tested. The COD removal from supernatant was primarily due to biological degradation

in the bioreactor while COD removal in the permeates were due to membrane filtration and biofilm (biofouling layer) on the membrane surfaces [27].

4.2. Membrane Resistance Profiles

The values of R_m , R_p and R_c and their relative percentages in R_t for submerged membrane process are presented in Table 2. UP 005 and UC 005 membranes had higher R_t values which were $13.52 \times 10^{12} \text{ m}^{-1}$ and $11.55 \times 10^{12} \text{ m}^{-1}$ than the other membranes. When the resistances compared for UP and UC, UC membranes had the greatest cake resistance except of UC 005 ($9.25 \times 10^{12} \text{ m}^{-1}$, $6.23 \times 10^{12} \text{ m}^{-1}$, and $4.92 \times 10^{12} \text{ m}^{-1}$, respectively). However, UC 010 had greatest pore resistance ($0.41 \times 10^{12} \text{ m}^{-1}$)

Table 2: Estimated Resistances Due to Membrane (R_m), Pore Blockage (R_p), Cake Formation (R_c) and Total Resistance (R_t)

Membrane type	$R_t (\times 10^{12})$ (m^{-1})	$R_m (\times 10^{12})$ (m^{-1}) (%)	$R_p (\times 10^{12})$ (m^{-1}) (%)	$R_c (\times 10^{12})$ (m^{-1}) (%)
UC 005	11.55	1.92 (16.6)	0.38 (3.3)	9.25 (80.1)
UC 010	6.80	0.16 (2.4)	0.41 (6.0)	6.23 (91.6)
UC 030	6.36	1.15 (18.1)	0.29 (4.5)	4.92 (77.4)
UP 005	13.52	1.44 (10.6)	0.48 (3.5)	11.60 (85.8)
UP 010	5.99	0.16 (2.7)	0.56 (9.3)	5.27 (88.0)
UP 020	4.87	0.96 (19.7)	0.19 (3.9)	3.72 (76.4)

compare to the others. Similar trend was observed for UP membranes in cake ($11.60 \times 10^{12} \text{ m}^{-1}$, $5.27 \times 10^{12} \text{ m}^{-1}$, and $3.72 \times 10^{12} \text{ m}^{-1}$, respectively) and pore resistances ($0.56 \times 10^{12} \text{ m}^{-1}$). For submerged membrane process, cake resistance had bigger values than pore resistance. This can be attributed to that as the pore size decreased the larger microbial aggregates could not pass through the surface pores of the membranes. However, the membranes with larger pore sizes (UC 010 and UP 010), the pore blockage resistance was estimated to be higher than the membrane resistance, indicating that some larger particles (or molecules) could enter the membrane matrix but could not to move through it. Increase in R_c was a result of accumulation of soluble fraction of microbial products (carbohydrate and protein) on the membrane surface.

4.3. Flux Analysis of the UF Membranes

The flux values of submerged membrane process for UC and UP membranes with various MWCO are shown in Figure 2 as a function of the time. The initial and pseudo steady-state flux values for each membrane are also presented in Table 3. Initial sharp

drop occurred for all the ultrafiltration membranes in the permeate flux within first twenty minutes. The flux gradually reduced after the initial drop and reached a pseudo steady state condition within 1,200 min. Steady state permeate flux values for UC with MWCO 5, 10, 30 kDa were 5, 7 and 9 L/m²/h, respectively. However, steady state permeate flux values for UP with MWCO 5, 10, 20 kDa were 4, 10 and 12 L/m²/h, respectively (Table 3). UP 20 kDa membrane yielded the greatest steady state flux value followed by UP 10 kDa, UC 30 kDa and UC 10 kDa, while UC and UP 5 kDa membranes had the lowest steady state flux value. The difference between UC and UP 5 kDa membranes was relatively less (UP 020 > UP 010 > UP 005 and UC 030 > UC 010 > UC 005). However, the greatest steady-state flux of UP 020 when compared the type of membrane material can be explained as a result of lowest contact angle after the filtration (Table 4). The critical flux and chemical cleaning-in-place (CIP) in a long-term operation of a pilot-scale submerged membrane bioreactor for municipal wastewater treatment were investigated by Wei *et al.* [28]. It was reported that the analyses from fourier transform infrared spectrometry (FTIR) with attenuated total

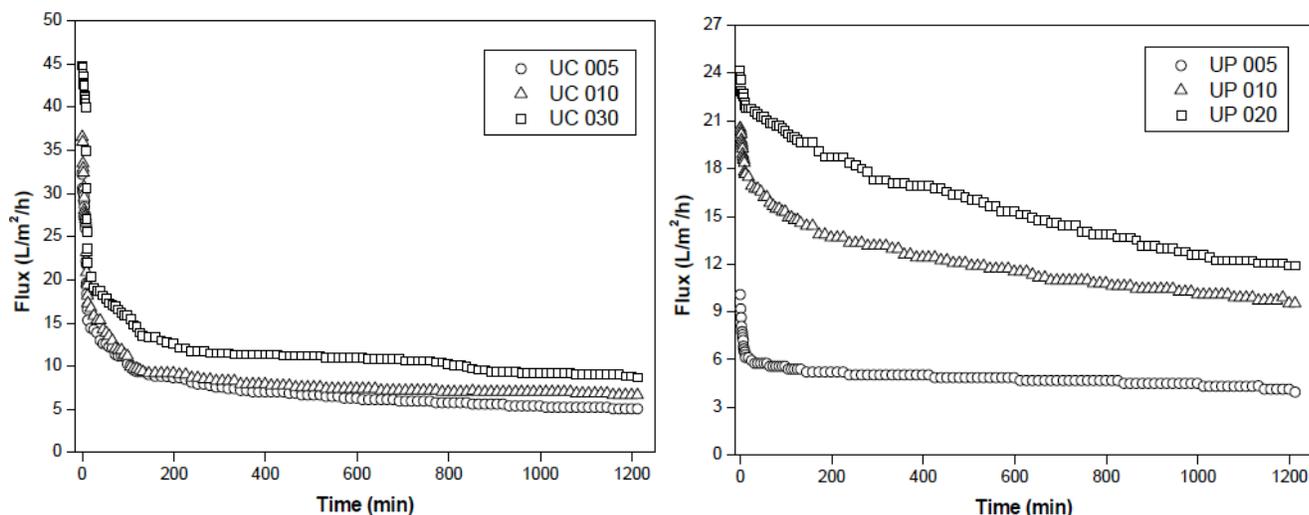


Figure 2: Variation of fluxes with time at different membranes with a different MWCO.

Table 3: Values of Initial and Steady-State Flux for Different UF Membranes

Membrane type	Initial flux (J_0) (L/m ² /h)	Steady-state flux (J) (L/m ² /h)
UC 005	32	5
UC 010	37	7
UC 030	45	9
UP 005	20	4
UP 010	21	10
UP 020	24	12

Table 4: Contact Angle Values of Unfouled and Fouled Membranes

Membrane type	Unfouled membranes	Fouled membranes
UC 005	10 <	31
UC 010	10 <	44
UC 030	10 <	57
UP 005	57	38
UP 010	45	47
UP 020	38	29

reflectance accessory (ATR) and energy dispersive spectrometer (EDS) demonstrated that protein-like macromolecular organics and inorganics were the important components of the fouling layer [28].

4.4. Morphological Characteristics

The surface roughness is an important parameter for membrane studies and it may influence the degree to which the foulants interact with membrane surface [29].

The AFM images of unfouled (Figure 3a-b) and fouled (Figure 3c-d) UC 010 and UP 010 membranes for submerged membrane process are presented in Figure 3a-d, respectively. Significant changes in surface morphology were observed for both types of membranes. The mean roughness (R_a) of membrane surface are presented in Table 5. The mean roughness of the UC membranes with MWCO increased by about 10 fold. For the UP membranes, the roughness increased by over 20 fold.

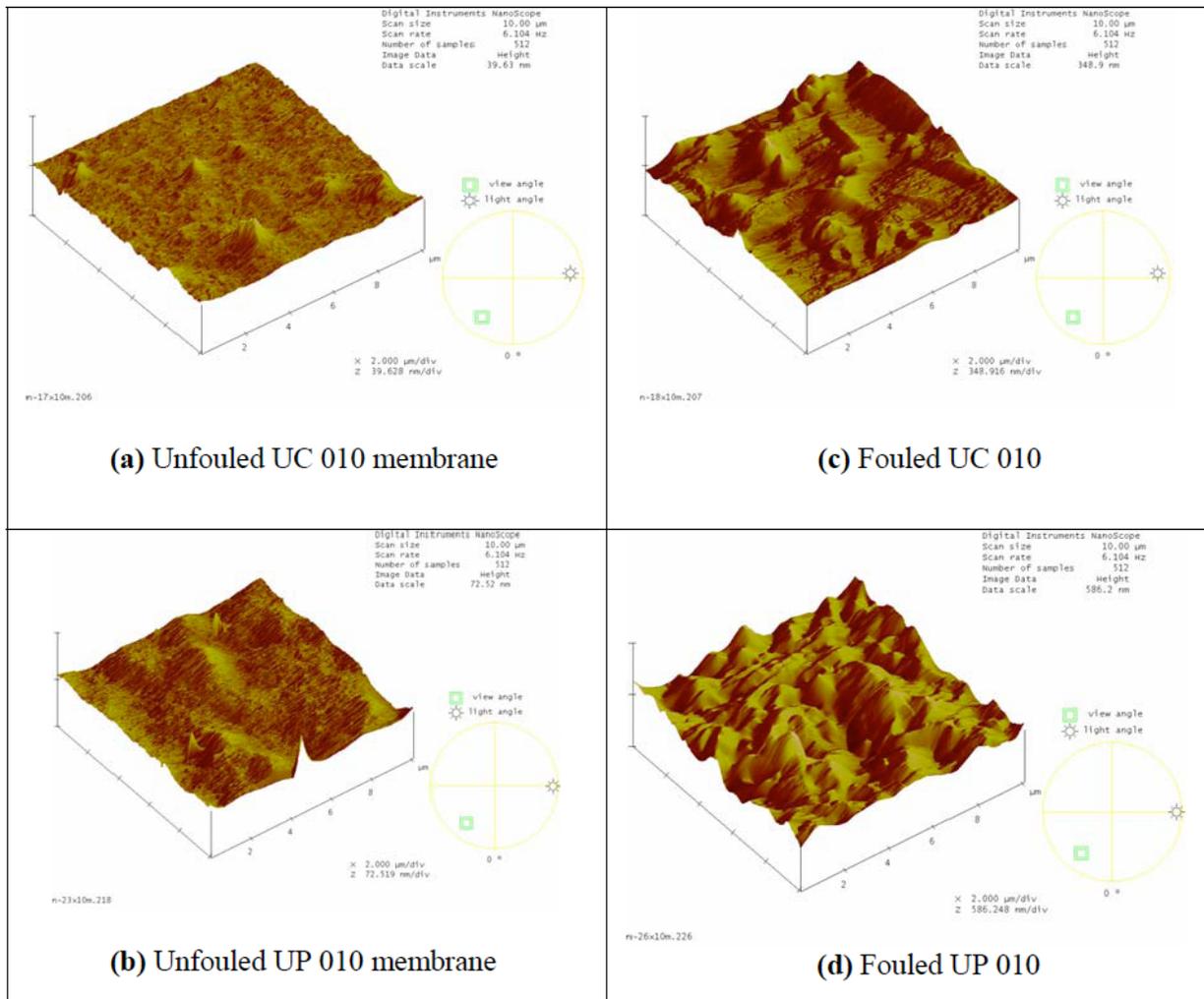
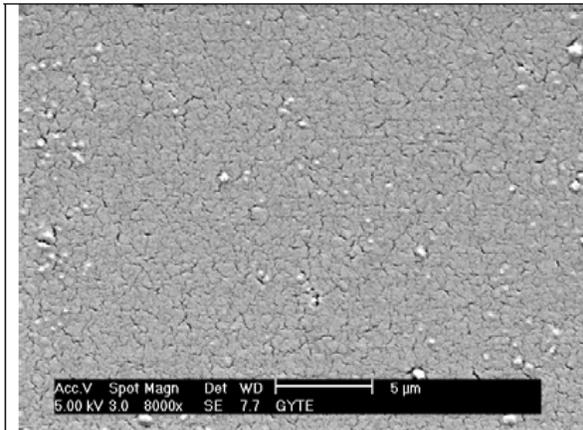
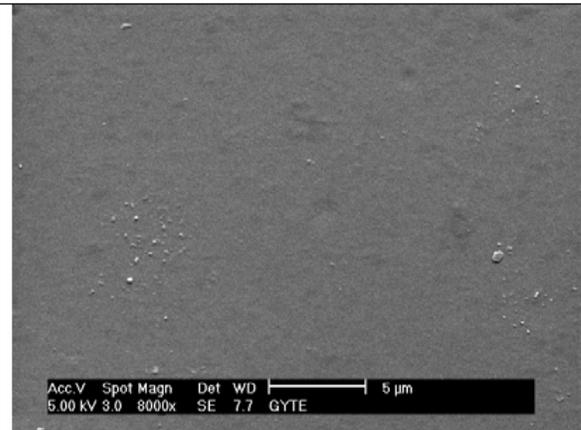
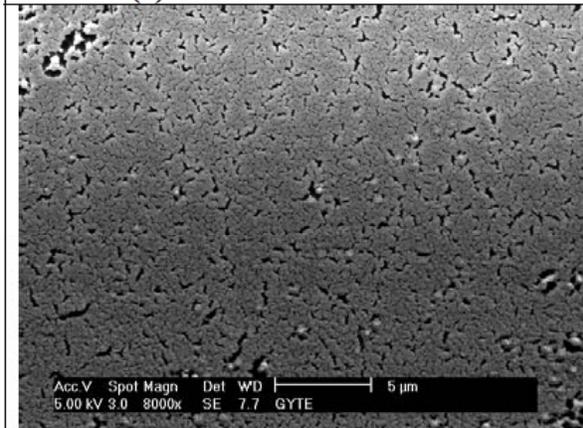
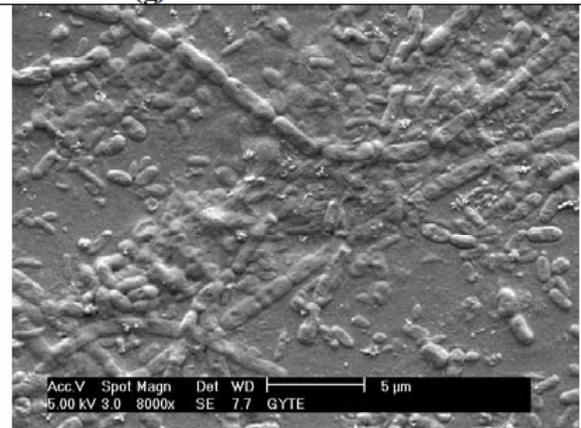
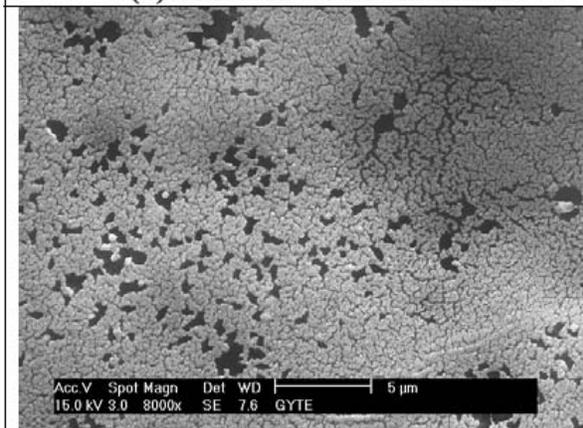
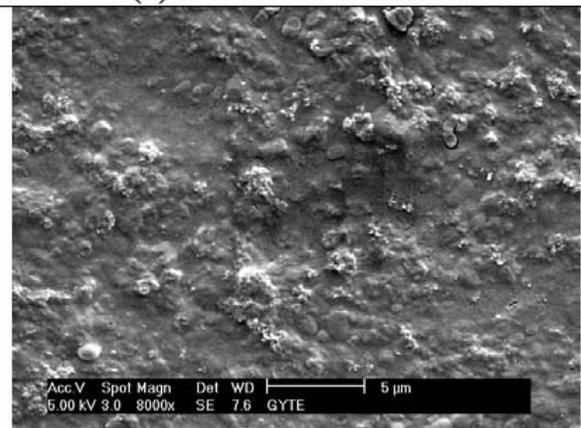
**Figure 3: AFM images of the unfouled (a-b) and fouled (c-d) UC and UP membranes.**

Table 5: Mean Roughness (R_a) of Unfouled and Fouled Membranes as Determined by AFM (Scanning of 10.0 μm x 10.0 μm Area)

Membrane type	Unfouled membranes	Fouled membranes
UC 005	2.02	29.74
UC 010	1.17	10.01
UC 030	4.84	55.12
UP 005	1.64	52.71
UP 010	2.09	30.76
UP 020	1.51	32.59

**(a) Unfouled UC 005 membrane****(g) Fouled UC 005 membrane****(b) Unfouled UC 010 membrane****(h) Fouled UC 010 membrane****(c) Unfouled UC 020 membrane****(i) Fouled UC 020 membrane**

(Figure 4). Continued.

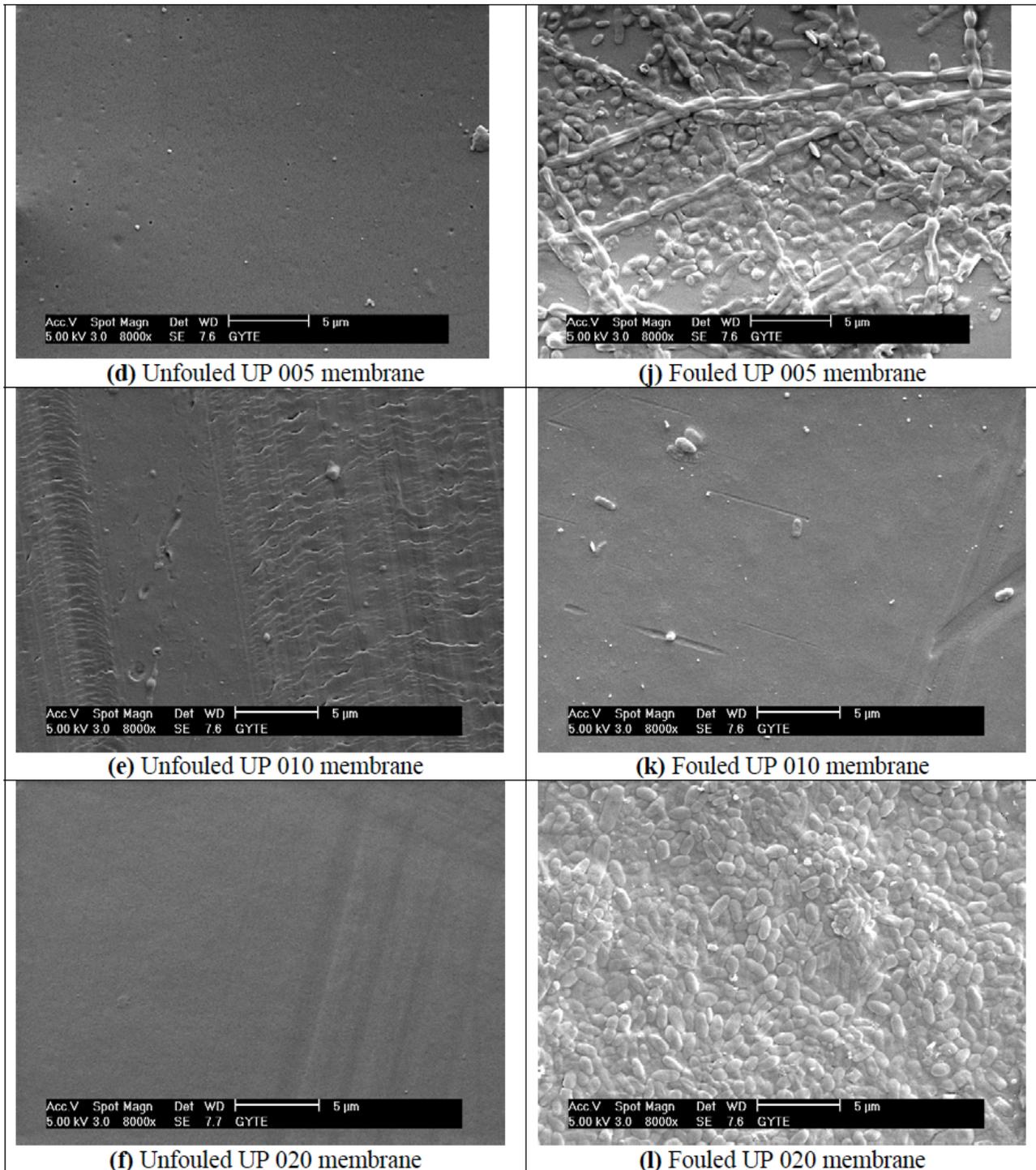


Figure 4: SEM images of unfouled (a-f) and fouled (g-l) UF membranes.

The SEM images of unfouled (Figure 4a-f) and fouled (Figure 4g-l) UC and UP membranes which belongs to submerged membrane process are shown in Figure 4a-l. The used membranes has a well defined layer of microorganisms accumulated on the surface (Figure 4g-l). The membrane fouling can be explained due to the formation of the gel layer which is caused by

deposition of floc forming bacteria on the membranes and results into significant flux reduction [30].

5. CONCLUSIONS

A laboratory scale MBR was used to investigate the performance of submerged UF membrane for removing

of soluble microbial products (SMP) (as proteins and carbohydrates) and fouling mechanisms. The COD concentration of the filtrates from both the UC and UP membranes showed a direct correlation with increasing MWCO. There was no significant difference in the total SMP removal effectiveness for both the cellulose (UC) and polyethersulphone (UP) membranes with different MWCO characteristics. However, UP membranes were relatively more effective in removing soluble carbohydrates, while UC membranes were more effective in removing soluble proteins. Analysis of the membrane performance data by resistances-in-series model indicated that cake fouling was the dominant membrane fouling mechanisms. Morphological examination of the membranes by SEM and AFM showed significant accumulation of microorganisms on the membrane surface.

ACKNOWLEDGEMENT

This study was financially supported by the TUBITAK, the Scientific and Technological Research Council of Turkey (Project No: 108Y129).

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Received on 07-01-2013

Accepted on 30-04-2013

Published on 31-05-2013

DOI: <http://dx.doi.org/10.6000/1929-6037.2013.02.02.6>