mechanisms consist of the adsorption of soluble and suspended extracellular polymers (EPS) and nutrients on a) the membrane surfaces and in membrane pores, b) the clogging of membrane pore structure by fine colloidal particles and cell debris, and c) the adhesion and deposition of sludge cake on membrane surfaces. Once microorganisms attach on the membrane surface, they grow and multiply, which leads to an increase in the amount of EPS. Biofouling can occur occasionally even in the extremely oligotrophic environment in which microorganisms can live with very low levels of nutrients (Flemming et al., 1997; Speth et al., 2000).

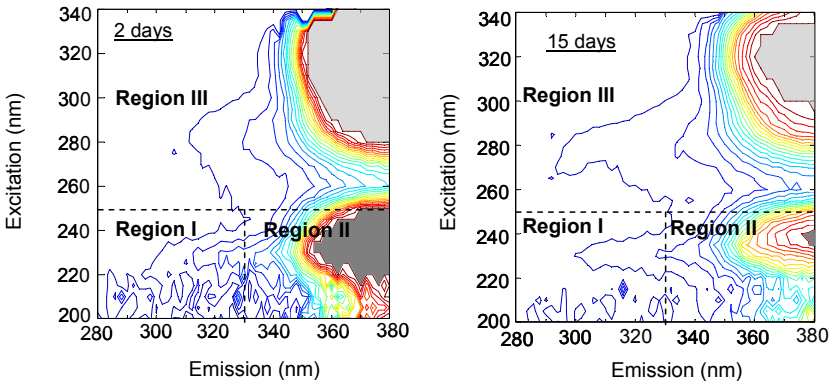


Figure 16.19 EEM fluorescence spectra of membrane foulants collected on 2 days and 15 days of operation (adapted from Aryal et al., 2009).

The biofilm plays a role as a trap for other particulate matter which builds up as a biomass. When a virgin membrane is exposed to a liquid environment containing microorganisms, dissolved organics and nutrients, the membrane will be covered with a layer of organic molecules due to the applied pressure, which is called 'conditioning layer'. The spiral-wound RO membrane offers an ideal environment for the formation of biofims on the membrane surface and on the spacer in the feed channel due to its confined mechanical design and complex fluid patterns around them. These biofilm layers vary with temperature, nutrient, pollution, and the depth of intake feed water (Saeed et al., 2000). The following mechanism is the attachment of suspended microorganisms on the membrane surface. Cell-surface interactions such as hydrophobic and electrostatic interaction will be affected. After the settlement of microorganisms, they grow and form the biofilm (Herzberg and Elimelech, 2007; El Aleem et al., 1998). Biofilm formation will be created by attached microorganisms and their utilization of food from the intake water or organics rejected by the membrane for cell growth and synthesis of the EPS matrix (Schneider et al., 2005).

The characterization of membrane biofouling is more complex than that of physico-chemical fouling because living microorganisms associated with the biofilm need to be handled. In addition, the biofouling in porous and nonporous membranes

is different. Since nutrients easily pass through MF and UF membranes, adhesion and biofilm formation on the filtrate surfaces of MF and UF membranes could not be expected to be more severe than that on the permeate surfaces of NF and RO membranes (Ridgway and Flemming, 1996). This leads to two different approaches of membrane biofouling characterization, direct and indirect methods. The indirect methods of biofouling characterization are i) flux decline, pressure difference, solute transport and ii) enumeration of greater numbers of bacteria in the brine. However, these methods cannot represent only biofouling. Direct methods are similar to characterization methods of physico-chemical fouling as described above such as SEM/EDX, AFM, DOTM, ICP/MS, FTIR, EEM, NMR, etc.. Schulenburg et al. (2008) reported that the NMR microscopy can be used to analyze and extract the spatial biofilm distribution, the velocity field and distributions of molecular displacement of passive tracers during biofouling of both industrial-scale spiral wound membrane modules. Among these methods, direct microscopic observation of membrane surfaces is known to be the most reliable and conclusive method for characterizing microbial communities and biofilm development (Ridgway and Flemming, 1996).

16.2.3.1 Confocal Laser Scanning Microscopy (CLSM)

CLSM is a technique which obtains high-resolution optical images with depth selectivity. It is widely used in numerous biological science disciplines, from cell biology and genetics to microbiology and developmental biology because it allows the non-destructive in-situ examination of biofilms on the membrane surface and can be effectively used for their visualization and quantification when combined with the application of a fluorescent probe (Yun et al., 2006). In a confocal laser scanning microscope, a laser beam passes through a light source aperture and then is focused by an objective lens into a small focal volume within or on the surface of a membrane. Scattered and reflected laser light as well as any fluorescent light from the illuminated spot is then re-collected by the objective lens. A beam splitter separates off a portion of the light into the detection apparatus, which also has a filter that selectively passes the fluorescent wavelengths while blocking the original excitation wavelength. After passing a pinhole, the light intensity is detected by a photodetection device, transforming the light signal into an electrical one that is recorded by a computer.

Figure 16.20 shows the CLSM images of biofilms formed on the hollow fiber MF membrane (hydrophilic polyethylene type) under aerobic and anoxic operational conditions with a synthetic dye wastewater. The biofilm was stained with SYBR Green I (Molecular Probes, Eugene, USA) specific to nucleic acid. The structure change of biofilm is clearly shown in terms of textural and volumetric parameters with the growth of the biofilm. The applied pressure continuously increases with the growth of the biofilm on the membrane in actual MBR operation under a constant flux. As such, the change in biofilm structure with growth was monitored in terms of textural and volumetric parameters.

16.2.3.2 Isolation and Identification of Microorganisms

In order to identify microorganisms on a biofouled membrane surface, the most simple and conventional method is to obtain a pure culture. This can be conducted by the streak-plate method. Microorganisms scraped from a biofouled membrane surface are spread over the surface of an agar (growth medium) plate by continuous dilution, so that the cells will be separated from each other. When the plate is incubated, those individual cells will grow into colonies that originats from a single cell. However the disadvantage of this technique is that some microorganisms may not grow on an agar plate.

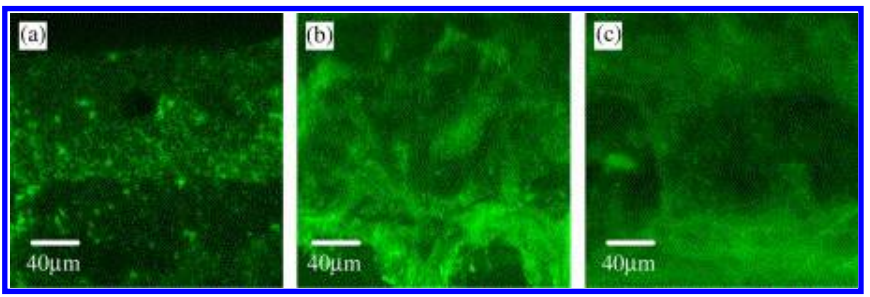


Figure 16.20 CLSM images of biofilms formed on the hollow fiber: (a) aerobic MBR at 2 kPa, (b) aerobic MBR at 30 kPa, (c) anoxic MBR at 30 kPa (adapted from Yun et al., 2006).

The identification of microorganisms of the bacterial communities on the biofouled membrane surface can be investigated using both polymerase chain reaction (PCR) denaturing gradient gel electrophoresis and 16S rDNA gene clone library methods after deoxyribonucleic acid (DNA) extraction with associated phylogenetic tree information. Extraction of DNA is often an early step in many diagnostic processes used to detect microorganisms. The procedure of DNA extraction is that the DNA of microorganisms scrapped from the fouled membrane is extracted by sonicating or bead beating. The addition of a detergent is necessary to remove lipid microorganism cells. DNA associated proteins, as well as other cellular proteins, are degraded with the addition of a protease. The protein is precipitated by the addition of a salt such as ammonium or sodium acetate. When the microorganisms on the fouled membrane are vortexed with phenol-chloroform and centrifuged, the proteins will remain in the organic phase. DNA is precipitated by mixing with cold ethanol or isopropanol and then centrifuged. The DNA is insoluble in the alcohol and will come out of solution, and the alcohol serves as a wash to remove the salt previously added. After pouring the alcohol off the pellet and drying, the DNA can be re-suspended in a buffer solution. The presence of DNA can be confirmed by electrophoresing on an agarose gel containing ethidium bromide, or another fluorescent dye that reacts with the DNA, and checking under UV light.

After DNA extraction, PCR amplification for 16S rRNA can be performed using a primer such as 9F (5'-GAG TTT GAT CCT GGC TCA G-3') and 1512R (5'-ACG GCT ACC TTG TTA CGA CTT-3') (Weisburg et al., 1991). The PCR mixture includes PCR premix, each primer and template DNA. The amplicons are purified using a PCR purification kit and the purified PCR products are ligated into a cloning vector and transformed into cells. Transformants are selected on an agar plate. A single white colony having the recombinant plasmids is inoculated into broth with ampicilin, and incubated. The plasmid DNA is then extracted and purified using the plasmid extraction kit. Clones can be sequenced using an automated DNA analyzer. Sequences can be interpreted using the BLAST database. Phylogenetic analyses of the cloned nucleotide sequence can be carried out using MEGA (Kumar et al., 2004). The tree is constructed from a matrix of pair-wise genetic distances by the neighbor-joining method.

Chon et al. (2009) investigated bacterial communities on fouled polyvinylidene fluoride MF membranes used in drinking water treatment over an 18 month period. They used the 16s rRNA gene clone library method which was constructed for the fouled MF membrane. Phylogenetic analyses of 120 randomly selected clones revealed 15 operational taxonomic units (OTU) assigned to various bacterial divisions (Figure 16.21). The results show that the proteobacteria dominated on the membrane surface, where the α-proteobacteria subdivision was the largest bacterial group (68% of total clones) and the β-proteobacteria subdivision was the second largest bacterial group (16% of total clones) found in the samples. The majority of the α-proteobacterial OTUs were primarily affiliated with *Bradyrhizobium* sp., *Rhodopseudomonas* sp. and *Afipia* sp. The remaining clones in this group were closely related to *Xanthobacter* sp. and *Agrobacterium* sp. In the β-proteobacteria, the most dominant OTU (11.8 % of total clones) was closely related to *Ralstoina* sp., while the other 3 OTUs were closely related to *Alcaligenes* sp., *Curvibacter* sp. and *Janthinobacterium* sp.

Table 16.12 shows various microorganisms that were identified on the biofouled membrane surface from different studies. Ridgway and Flemming (1996) reported that biofouling on the membrane surface occurs in less than two weeks during actual membrane operation. However, it should be noted that the microorganisms identified from the fouled membrane are different as a function of feed solution, membrane type, season, operational time, etc. For example, Ho et al. (1983) reported that the most predominant species on the fouled RO surface are *Penicillium, Fusarium, Pseudomonas, Bacillus* and *Lactobacillus.* On the other hand, *Acinetobacter, Flavobaterium/Moraxella, Pseudomonas/Alcaligenes* are predominant (Ridgway et al., 1981). This suggests that most of the various microorganisms promote biofilm formation and have significant potential to cause biofouling.

16.2.3.3 Flow Field-flow Fractionation (FlFFF)

The principle of FlFFF has been explained in Section 16.2.2.2. Membrane biofouling caused by bacteria that have different characteristics has been recently

evaluated using FlFFF (Lee et al., 2010; Lim et al., 2010). Three different bacteria with different size and shape (*S. epidermidis, E. coli, F. lutescens*) were investigated with GM UF (rough with a low negative surface charge and relatively high hydrophobicity) and NE70 NF (smooth with a high negative surface charge and relatively low hydrophobicity) membranes. Figure 16.22 shows retention time distributions of various

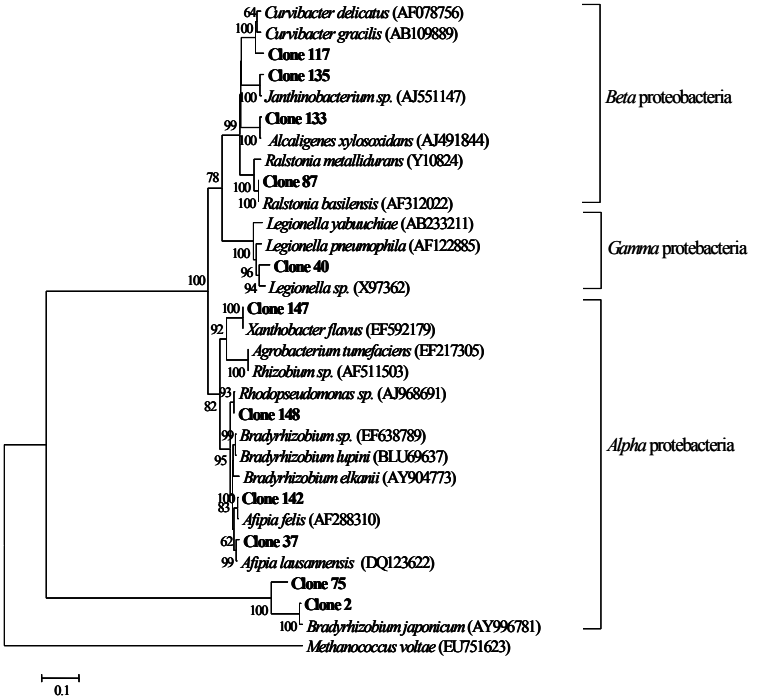


Figure 16.21 Phylogenetic tree of the proteobacteria based on 16S rDNA sequences obtained from cloning analyses of a fouled hollow fiber MF membrane. Clones obtained from this study are shown in *bold type.* The tree was derived by performing the neighbor-joining method with a Jukes-Cantor parameter in the MEGA version 3.1 programme. Bootstrap values greater than 50% (1000 replicates) are shown. *Methanococcus voltae* EU751623 was used as the out-group to root the phylogram. The scale bar represents one substitution per ten nucleotides.

bacteria on UF membranes with different carrier solutions with de-ionized water and 10 mM KCl. The size of the tested bacteria measured using the FlFFF was compared to those of the reference particles and the size determined from TEM images. The size (in μm) of *S. epidermidis*, *E. coli* and *F. lutescens* was 0.5, 1.5, 2.5 by TEM and 0.8, 2.6, 1.8 by FIFFF. The size of *E. coli* determined from TEM images was different from that by FlFFF, which increased from 1.5 μm to 2.6 μm, suggesting that *E. coli* was preferentially retained on the membrane surface. The FlFFF retention

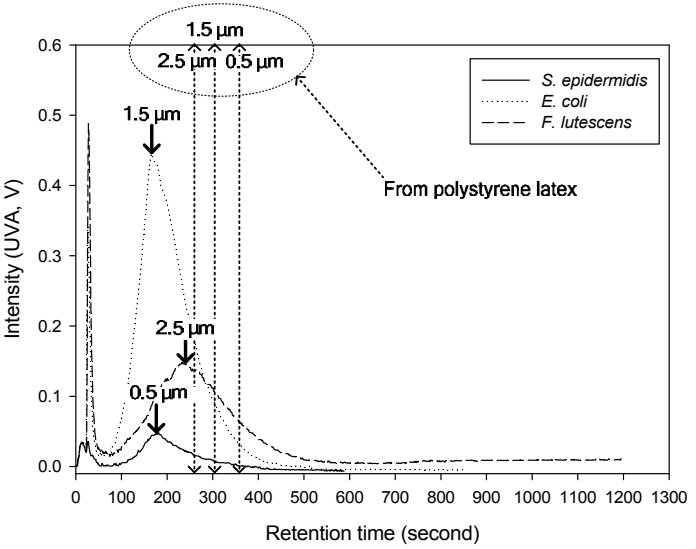
time of *S. epidermidis, E. coli* and *F. lutescens* was highly influenced by the ionic strength of the solution and the surface polarity of the membranes and bacteria. The NF membrane was found to have a higher potential of biofouling than the UF membrane with the bacteria tested in this study. *E. coli* was the most significant biofoulant among the bacteria tested on both membrane surfaces based on FlFFF retention times compared to other bacteria.

Table 16.12 Microoganisms isolated from membrane biofouling.

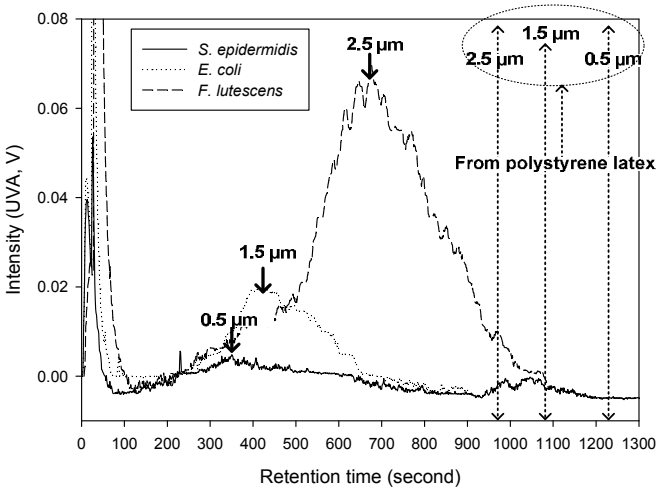
Membrane/water	Microorganism identified	Reference
RO/canal	*Pseudomonas (not aeruginosa, Pseudomonas fluorescens*	Sinclair, 1982
Sand filter + RO/brackish groudwater	*Fungal: Acrmonium, Candida, Cladosprorium, Cleistothecial ascomycetes, Fusarium, Geotrichum, Mucorales, Mycelia sterilia, Penicillium, Rhodotorula, Trichoderma Bacteria: Acinetobacter, Arthrobacter, Bacillus, Flavobacterium, Kurthia, Lactobacillus, Micrococcus, Micromonospora, Pseudomonas,*	Ho et al., 1983
RO/groundwater	*Seliberia*	Kutz et al., 1986
RO/ reservoirs in households	*Pseudomonas (not aeruginosa), Alcaligenes or Moraxella, Acinetobacter, Flavobacterium, Chromobacterium*	Payment, 1989
RO and NF/synthetic wastewater	*Flavobacterium, Pseudomonas, Ralstonia, Cytophaga, Verrucomicrobia, Planctomycetes*	Ivnitsky et al., 2005
Flocculation + MF/drinking water	*Bradyrhizobium, Rhodopseudomonas, Afipia, Xanthobacter, Agrobacterium, Ralstoina, Alcaligenes, Curvibacter, Janthinobacterium, Planctomycetes, Bacteroidetes, Legionella, Arthrobacter, Marinicola seohaensis*	Chon et al., 2009
RO/drinking water	*Trinema, α, β and γ-Proteobacteria, Cytophaga, Flavobacter, Bacteroidetes, Actinobacteria, Sphingopyxis, Burkholderiales, Verrucomicrobia.*	Bereschenko, et al., 2010

16.3 Concluding Remarks

Membrane technology is the most reliable option to separate unwanted materials for drinking water, industry water, wastewater reuse and desalination. However, the technology faces a major obstacle which is membrane fouling. Membrane fouling includes complex mechanisms and foulants. To resolve the membrane fouling, better understanding and characterization of fouling will significantly lead to improving fouling prevention.



(a) De-ionized water



(b) 10 mM KCl

Figure 16.22 Retention time distributions of various bacteria on GM membranes with different carrier solutions. (a) De-ionized water, and (b) 10 mM KCl.

The characterization of membrane foulants as a diagnostic tool consists of physical, chemical and biological analyses. The physical analyses of fouled membrane surface provide valuable information on structure, roughness, charge effect, strength and hydrophobicity of membrane fouling. The chemical properties imply qualitative and quantitative measurements of different inorganic and organic matter including elemental investigation of different foulants. The biological characteristics give the spatial biofilm distribution, structure of dominant microorgnisms and isolation and identification of microorganisms. These characterization techniques are also related to many different foulant types such as structure, roughness, hydrophobicity, charge effect, strength, calcium, magnesium, aluminum, iron, silicate, particle, functional group, biopolymer, humic acid, polysaccharide, structural composition, biofilm structure, microorganism and foulant interaction.

Based on the detailed characterization of membrane fouling, the multi-angle information implements the appropriate selection of different pretreatments and the best optimization of the correct design operating parameter.

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