Do biological-based strategies hold promise to biofouling control in MBRs?

Lilian Malaeb a, Pierre Le-Clech b, Johannes S. Vrouwenvelder a, c, d, George M. Ayoub e, Pascal E. Saikaly a, *

a Water Desalination and Reuse Research Center and Division of Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology, Thuwal 23955-6900, Saudi Arabia
b UNESCO Centre for Membrane Science and Technology, School of Chemical Engineering, The University of New South Wales, Sydney, NSW, Australia
c Department of Biotechnology, Faculty of Applied Sciences, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands
d Wetsus, Centre of Excellence for Sustainable Water Technology, Agora 1, 8900 CC Leeuwarden, The Netherlands
e Department of Civil and Environmental Engineering, American University of Beirut, Beirut, Lebanon

Abstract

Biofouling in membrane bioreactors (MBRs) remains a primary challenge for their wider application, despite the growing acceptance of MBRs worldwide. Research studies on membrane fouling are extensive in the literature, with more than 200 publications on MBR fouling in the last 3 years; yet, improvements in practice on biofouling control and management have been remarkably slow. Commonly applied cleaning methods are only partially effective and membrane replacement often becomes frequent. The reason for the slow advancement in successful control of biofouling is largely attributed to the complex interactions of involved biological compounds and the lack of representative-for-practice experimental approaches to evaluate potential effective control strategies. Biofouling is driven by microorganisms and their associated extra-cellular polymeric substances (EPS) and microbial products. Microorganisms and their products convene together to form matrices that are commonly treated as a black box in conventional control approaches. Biological-based antifouling strategies seem to be a promising constituent of an effective integrated control approach since they target the essence of biofouling problems. However,
1. **Biofouling problem in MBRs**

Membrane bioreactors (MBRs) are now broadly applied as wastewater treatment technology that combines membrane processes and suspended growth bioreactors. Compared to other technologies e.g. activated sludge processes (Kim et al., 2010; Judd, 2008; Visvanathan et al., 2000), MBRs allow smaller footprints, higher-quality effluents, less sludge and complete solid–liquid separation; however, membrane fouling remains in most cases a persistent problem (increasing operating costs and reducing the water quality and quantity) (Wang et al., 2009; Kimura et al., 2009). Fouling results in a lower permeate flux, higher trans-membrane pressure (TMP), frequent membrane cleaning or replacement and overall degraded membrane performance (Mahendran et al., 2011; Liao et al., 2004). Fouling can be organic, inorganic or biological, although the boundary between these classifications is not rigid and the definitions of different fouling types may overlap. For example, inorganic deposition can be a direct consequence of biologically-induced mineralization between biopolymers and salts (Wei et al., 2007; Herrera-Robledo et al., 2011) and internal fouling caused by the adsorption of
dissolved organic and inorganic matter into membrane pores in MBRs is known to occur simultaneously with biofouling (Yamato et al., 2006; Rosenberger et al., 2006; Miura et al., 2007).

Among all types of fouling, biofouling is a dynamic, complex and relatively slow process that involves interactions not yet thoroughly understood (Pasmor et al., 2001; Baker and Dudley, 1998). While it is possible to conduct controlled experiments on other foulants, the enormous diversity of bacterial communities and their close association with excreted extracellular polymers (EPS) and soluble microbial products (SMP) complicate experimental approaches that would help explicate this challenging phenomenon. Biofouling occurs as a result of two mechanisms: (i) colonization of membrane surfaces with microorganisms and (ii) production of membrane foulants by microorganisms in the mixed-liquor. Both the microbes and their products contribute to membrane fouling, as evidenced by studies that show enhanced membrane permeability due to quorum quenching, which targets the microbes (e.g. Yeon et al., 2009a,b; Oh et al., 2012; Jiang et al., 2013), and studies that correlate fouling to EPS/SMP (e.g. Hwang et al., 2008; Nataraj et al., 2008; Wang et al., 2009; Kimura et al., 2009). With the currently used analytical approaches, it is still challenging to determine which of the two mechanisms is dominant and under which conditions. However, with advances in analytical characterization tools, it becomes possible to conduct systematic studies that elucidate the key players in the biofouling process and quantitatively correlate the characteristics of both the microbial communities and their products to biofouling rates.

The importance of biofouling relative to other fouling mechanisms is also ambiguous. Some researchers report that sludge-cake resistance is much higher than pore blocking resistance (Lee et al., 2001; Eusebio et al., 2010; Pendashteha et al., 2011) and others consider the cake layer to be a removable type of fouling while pore blocking as non-removable by physical cleaning and hence requiring chemical methods (Meng et al., 2009). Wu et al. (2011) assumed cake resistance to be due to suspended solids whereas pore constriction and blocking to be caused by solutes and colloids, respectively. The accuracy of these divisions and of hypothetical biofouling models, however, is questionable due to the complex interactions among foulants as well as to differences in process configuration, operating conditions and extraction methods.

The effects of operational and wastewater characteristics have also been considered but discrepancies are commonly noted among different studies. Research findings on the effect of sludge retention time (SRT) are largely inconsistent (Meng et al., 2009; Sweity et al., 2011). Increasing the SRT generally leads to a better filterability (Nguegijimong et al., 2005; Trussell et al., 2006; Liang et al., 2007); however, a further increase can intensify fouling (Han et al., 2005). Rosenberger and Kraume (2002) found that specific SMP concentration increased with sludge loading rate and decreased with sludge age. Although shear stresses can alleviate fouling, shear imposed on microbial flocs could result in the release of EPS (Wang et al., 2009) and high shear conditions associated with crossflow configuration can induce release and accumulation of soluble organic matter in the mixed liquor, which increases its fouling propensity (Stricot et al., 2010). Fouling is also reported to increase with higher food to microorganisms ratio (F/M) (Trussell et al., 2006) yet the mechanisms of these effects remain to be clarified particularly in relation to floc characteristics, which are in turn related to EPS (Jang et al., 2006). Zhang et al. (2010) noted that during the start-up period, MBR fouling is higher for variable than for constant organic loading but after 2 SRTs, less fouling occurs with variable loading. A high ratio of monovalent over polyvalent cations also worsens filtration characteristics due to sludge deflocculation (Van den Broeck et al., 2010). Constant flux can prevent excessive fouling (Defrance and Jaffrin, 1999) but may lead to irreversible fouling (Ye et al., 2005; Le-Clech et al., 2006). Moreover, low fluxes cause much less homogenous live/dead cell profiles, presumably due to starvation and oxygen stress, which can increase cell membrane porosity, death and SMP release (Hwang et al., 2008; Drews, 2010).

The biofouling process, led by various microorganism types and their excreted products, develops into a complex and difficult to control problem, so that conventional physical cleaning processes such as back-washing and back-pulsing are no longer effective (Chu and Li, 2005; Le-Clech et al., 2006). Chemical cleaning also has limited success and microorganisms can adapt to cleaning procedures by changing their physiological responses through gene regulation and metabolism (Ma et al., 2009; Mahendran et al., 2011; Calderon et al., 2011). Other control approaches focus on membrane modification, so as to generate surfaces with lower fouling potential. Yet, the stability of most proposed polymers and functionalized surfaces is often not evaluated for long-term filtration where severe convective forces and cyclical cleaning protocols are applied (Mansouri et al., 2010).

Control strategies commonly used in practice tend to treat biofouling as a black box by ignoring its intrinsic biological nature. Recently, biological-based antifouling strategies started to prove their potential as a more viable solution than conventional control methods, based on engineering, material, and chemical principles (Yeon et al., 2009b). As a result, an integrated control approach that takes into account biological methods is potentially more effective for understanding and ultimately controlling this persistent problem that continues to defy MBR operators. In order to define further needed research efforts, an evaluation of MBR membrane biofouling is presented from the microbiological perspective. This critical review is divided into three distinct sections describing:

i. Limited effectiveness of conventional control methods and potential of biological-based strategies in offering better antifouling alternatives;
ii. Analytical tools presently used and insights from studies on microbial communities and their EPS for an improved understanding and management of the biofouling problem;
iii. Existing knowledge gaps and future research needs in terms of both microbiological investigations and biological-based control applications developed specifically for MBRs.

Finally, it is expected that an integrated approach that combines various successful strategies is more likely to be effective than any single antifouling method. Linking
microbiological/EPS studies to design and operational parameters that are representative of real-scale MBRs is crucial for the development of such an approach with the ultimate aim of effectively controlling MBR biofouling.

2. Current status of antifouling strategies

2.1. Commonly applied control strategies

Conventional physical (e.g. back-washing, back-pulsing, air sparging) and chemical (e.g. acids, bases, oxidants, chelating agents, polymeric coagulants, surfactants) cleaning methods often fail to adequately control biofouling. In the membrane-coupled upflow sludge blanket bioreactor analyzed by Calderon et al. (2011), backflushing and chemical cleaning using NaClO were found ineffective in completely removing the biofouled layers from MBR membranes and remaining populations supported rapid biofilm re-growth, leading to the regeneration of a similar microbial community structure. In fact, a biofilm formed under natural conditions may prove easier to control in the long-term than one formed where biocides are in use (Baker and Dudley, 1998). Bacteria surviving after a biocide application can develop defense mechanisms, which render them even more resistant to the biocide when reapplied later. Frequent chemical cleaning moreover reduces membrane lifetime (Judd, 2008), can impair permeate quality (Tao et al., 2005), and is associated with undesirable waste streams that have to be properly disposed of (Brepols et al., 2008). It was further found that declogging the membrane to remove particulate matter that fills void spaces in membranes can recover permeability for a short time but is followed by a rapid permeability decline due to aggravated fouling over the course of a few hours of time (Zsirai et al., 2012). Other control strategies to reduce fouling and improve flux consistency involve membrane modification (Table 1) but this also has limited applicability as it is difficult to modify membrane structures without occluding membrane pores or impacting the separation performance (Mansouri et al., 2010). In addition, many coatings do not show long-term mechanical or chemical stability. Membrane properties such as hydrophobicity, charge and roughness only affect the rate of early bacterial attachment and under the specific conditions tested and even the surfaces most resistant to biofilm formation have been reported to show a 40% surface coverage within 2 days (Fasmore et al., 2001).

It was suggested that alternative antifouling strategies directed towards microbial groups that have shown resistance to standard cleaning methods (e.g. members of the family Sphingomonadaceae and methanogenic Archaea) might be more promising (Calderon et al., 2011). In this regard, biological-based antifouling strategies might prove to be valuable and may potentially enhance the effectiveness of currently applied cleaning protocols. These strategies refer to the use of certain chemicals that target a specific molecule or mechanism that is responsible for the attachment, communication, motility or growth of microbial cells. In this paper, the definition of biological-based antifouling strategies is also expanded to include phages and predators, which respectively infect and consume microorganisms.

2.2. The potential of biological-based antifouling strategies

2.2.1. Biological-based biofilm control strategies

Among the commonly tested biological strategies for biofilm control are quorum quenching (QQ), enzymatic disruption (ED), energy uncoupling (EU), cell wall hydrolysis and the use of microbial predation and bacteriophages. Biological-based strategies are reported to offer the advantages of higher efficiency, lower toxicity, more sustainability and less bacterial resistance over other control approaches (Yeong et al., 2009b; Xiong and Liu, 2010). QQ refers to the inhibition of the cell-to-cell communication known as quorum sensing (QS) that is used by microorganisms to coordinate their behavior e.g. biofilm formation, motility, EPS production and detachment.

Table 1 – Specific membrane modifications to control biofouling in MBRs.

<table>
<thead>
<tr>
<th>Membrane modifications and observations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original wastewater biofilms are highly tolerant to Ag–NP&lt;sup&gt;a&lt;/sup&gt; treatment but susceptibility is different for each microorganism e.g. Thiobacillus is more sensitive to Ag–NP than other bacteria.</td>
<td>Sheng and Liu (2011)</td>
</tr>
<tr>
<td>Modifying membranes to become hydrophilic or electrically charged only partially reduces biofouling.</td>
<td>Liu et al. (2019)</td>
</tr>
<tr>
<td>Acrylic acid grafted membrane showed better anti-fouling than acrylamide modified membranes and surface carboxyl-containing membranes were better than surface amido-containing membranes.</td>
<td>Li et al. (2010)</td>
</tr>
<tr>
<td>Ceramic membranes coated with fullerene C60&lt;sup&gt;b&lt;/sup&gt; reduced microbial attachment &amp; inhibited microbial respiratory activity.</td>
<td>Mansouri et al. (2010)</td>
</tr>
<tr>
<td>Surface modification of polypropylene hollow fiber MBR microporous membranes in MBR showed better anti-fouling efficiency as follows: graft polymerization of acrylic acid &gt; graft polymerization of acrylamide acid &gt; CO&lt;sub&gt;2&lt;/sub&gt; plasma treatment &gt; NH&lt;sub&gt;3&lt;/sub&gt; plasma treatment.</td>
<td>Yu et al. (2008)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ag–NP: silver nanoparticles.
<sup>b</sup> C60: fullerene.
Several signaling pathways have been identified for QS among microorganisms including acyl-homoserine lactone (AHL) signal genes, amino acid peptides, natural small molecules (NSM) that can affect EPS synthesis and biofilm formation (Lopez et al., 2009) and auto-inducers such as AI-2 signal. Another widespread signal for biofilm disassembly is a mixture of D-amino acids, which could disassemble biofilms in Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus by releasing amyloid fibers that link the cells together (Kolodkin-Gal et al., 2010). This could be a strategy used by biofilm bacteria to create local channels and facilitate mass transport within biofilms (Flemming and Wingender, 2010) and may also prove to be an effective biofilm control solution.

EPS-degrading enzymes that have been successfully applied for biofilm detachment include proteolytic enzymes for protein hydrolysis (e.g. proteinase K, trypsin and subtilisin), polysaccharases for the hydrolysis of polysaccharides (e.g. Dispersin B, Mutanase and dextranase) as well as DNases (Petersen et al., 2005; Chaignon et al., 2007; Leroy et al., 2008; Guezenec et al., 2012). Hydrolytic enzymes of cell walls such as the antimitotic protein lysozyme have been used to prevent microbial attachment and could act more specifically than traditional biocides (Xiong and Liu, 2010). Energy uncoupling via the addition of chemicals (e.g. 2,4-dinitrophenol DNP, carbonyl cyanide chlorophenylhydrazone CCCP, and 3,3',4',5-tetrachlorosalicylanilide TCS) can transport protons through the cellular membrane thus dissipating the proton gradient and inhibiting adenosine triphosphate (ATP) synthesis. Xu and Liu (2011) showed evidence of suppressed ATP synthesis and AI-2 production using DNP to remove biofilm bacteria from nylon membrane surfaces.

The use of bacteriophages to control biofilm growth is another promising strategy due to the specificity of these acellular microorganisms, their rapid multiplication and limited infectivity to prokaryotic organisms (Doolittle et al., 1996; Lu and Collins, 2007). Polysavent phages to prevent concomitant adhesion and biofilm formation have proved their effectiveness for multi-consortia (Jensen et al., 1998). Phage resistance through reversion, however, can be a challenge. On the other hand, some phages (e.g. polysavent phage K) have shown that continuous propagation through bacterial strains yields modified phages with enhanced lytic properties toward the strains (O’Flaherty et al., 2005). Other studies proved the ability of genetic engineered phages containing biofilm-degrading enzymes to degrade EPS (Lu and Collins, 2007). Predators, which reduce biomass concentration and alter its morphology due to grazing, motility or sloughing, have also been used. Derlon et al. (2012) investigated the impact of predation by eukaryotes on the development of biofilm structures in gravity-driven dead-end UF systems operated under ultra-low pressure conditions. They found that an open and heterogeneous biofilm structure developed in systems with predation, resulting in higher permeate fluxes.

2.2.2. Application to MBRs

To date, a limited amount of biological control strategies has been applied to membranes for water/wastewater treatment in general, or to MBRs in particular. Poole and van der Graaf (2005) used enzymatic cleaning by protease to remove protein biofouling in UF membranes, which resulted in a much higher efficiency compared to traditional alkaline cleaning. The first studies in MBRs were conducted by Yeon et al. (2009a,b), who demonstrated the potential of porcine kidney acylase I and AHL-acylase enzymes to prevent MBR biofouling by quenching AHL autoinducers. AHL-acylase directly immobilized onto a nanofiltration (NF) membrane was shown to prohibit the formation of mushroom-shaped mature biofilm due to reduced EPS secretion (Kim et al., 2011). To avoid practical issues of cost and stability of enzymes, Oh et al. (2012) proposed that QQ might be more feasible, has a longer life span and does not require enzyme purification. They encapsulated Recombinant Escherichia coli producing N-acyl homoserine lactonase or Rhodococcus sp. isolated from a real MBR inside microporous hollow fiber membrane and could efficiently control biofouling (Jahangir et al., 2012; Oh et al., 2012). The microbial-vessel maintained its QQ activity over 100 days of MBR operation due to continuous regeneration of living quorum, but the QQ effect was largely dependent on the recirculation rate of the mixed liquor between the bioreactor and the membrane tank (Jahangir et al., 2012). Higher recirculation rates facilitated transport of signal molecules from the biofilm into the bulk mixed liquor and then to the microbial-vessel (Jahangir et al., 2012). Jiang et al. (2013) showed that using porcine kidney acylase I enzyme inhibited biofilm formation and enhanced membrane permeability with no apparent effects on effluent quality of the lab-scale MBR that was fed with synthetic wastewater. In this study, QQ was found to increase settleability, reduce the production of polysaccharides and proteins and reduced viscosity and relative sludge hydrophobicity (Jiang et al., 2013).

Ngo and Guo (2009) found that a green bioflocculant from a natural starch-based cation reduces fouling in an aerated MBR but noted that further study on its sustainability in biological treatment is required. It was also suggested that selection pressure to enhance populations of Bacillus and Pseudomonas species for their enzymes that digest carbohydrates and proteins might be effective in reducing membrane fouling (Ng and Ng, 2010). As for energy uncoupling, no application has been conducted to MBR membranes. The study by Xu and Liu (2011), which used DNP to remove biofilms from nylon membrane surfaces is promising but was conducted for hydrophilic flat-sheet nylon membranes used in dead-end microfiltration (MF) filtration of batch-fed synthetic wastewater.

In terms of the use of bacteriophages, Goldman et al. (2009) reported that the addition of bacteriophages reduced by 40–60% the microbial attachment to UF membrane in MBR treating effluents containing three bacterial species. However, few phages (1–10 PFU/100 mL) were detected in the permeate, which can pose a problem in real applications. Moreover, a combination of several phages would be required to combat the multiple bacterial species encountered in real-scale systems.

As to biofouling control using predation, available studies are very limited. A metazoa population, primarily composed of rotifers and oligochaete worms has been shown to play an effective role in MBR biofouling control and reducing cake formation on the membrane (Luxmy et al., 2001); yet it was mentioned in this study that high mixed liquor suspended
solids (MLSS) in MBR might suppress the activity of these metazoa. Derlon et al. (2012) noted that predation is especially useful in systems operated without cross-flow that may provide suitable conditions for the larger eukaryotic organisms to develop (Derlon et al., 2012). Table 2 summarizes success stories of biological-based antifouling strategies as applied to MBRs in particular. The enhancement in permeability as reported in the corresponding studies is given in terms of trans-membrane pressure or permeability.

On the other hand, studies discussed in this section on the successful application of biological-based methods to reduce biofouling in membranes other than MBRs (Poole and van der Graaf, 2005; Goldman et al., 2009; Kim et al., 2011; Xu and Liu, 2011; Derlon et al., 2012) are also encouraging although the expansion to MBR conditions requires accounting for the more complex environment and parameters involved. To enhance the development and application of the above biological-based antifouling strategies in MBRs requires first understanding and characterizing the main elements responsible for biofouling. These include both the microorganisms and their associated EPS as discussed in the next section.

3. Implications from existing EPS/microbial studies

3.1. EPS studies in MBRs

Membrane modules and materials, hydrodynamic parameters, process and environmental conditions, and physicochemical properties of the treated water are among the dominant factors determining membrane biofouling (Liao et al., 2004). These factors may be divided into two categories: biomass and operational parameters. A summary of the impact of these parameters has been presented in several reviews (e.g. Le-Clech et al., 2006; Meng et al., 2009; Drews, 2010). Among the most important and complex biomass parameters is related to EPS, which is often differentiated into loosely bound, tightly bound and soluble EPS, also known as slime polymers (SMP). EPS consist of macromolecules of microbial origin (polysaccharides, proteins, glycoproteins, lipoproteins) (Flemming, 1997) and insoluble material (sheaths, capsular polymers, condensed gel, loosely bound polymers and attached organic material) produced by active secretion, shedding of cell surface material or cell lysis (Jang et al., 2005).

3.1.1. EPS extraction and characterization

EPS nature and quantity vary greatly among biofilms, depending on the microorganisms present, shear forces, temperature and nutrients while EPS identification largely depends on the isolation method (Flemming and Wingender, 2010). Extraction and characterization methods for EPS have been extensively reviewed in the literature (e.g. Zhang et al., 1995; Drews, 2010). The determination of EPS concentration mostly relies on measuring carbohydrates and proteins while different characterization methods can give different results. Some authors measure total or dissolved organic carbon (TOC/DOC) or chemical oxygen demand (COD) instead of measuring individual carbohydrate and protein fractions (Haberkamp et al., 2007; Wang et al., 2009); however, with both

<table>
<thead>
<tr>
<th>Method</th>
<th>Description of biological-based antifouling strategy used in MBR</th>
<th>Type of MBR [flux]</th>
<th>Enhancement of permeability in terms of TMP(^{a}) [compared to control MBR]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED</td>
<td>Bioassay with Agrobacterium tumefaciens A136 supplemented with spectinomycin and tetracycline to maintain two plasmids that provide the AHL response system</td>
<td>Batch MBR with total recycle mode [15 Lmh]</td>
<td>~32 h to reach 40 kPa (~20 h in control); maximum TMP 48 kPa at ~40 h [70 kPa at ~23 h for control]</td>
<td>Yeon et al. (2009a)</td>
</tr>
<tr>
<td>ED</td>
<td>Magnetic enzyme carriers prepared by immobilizing the QQ Porcine kidney acylase I on a magnetic carrier and recycled back from the redrawn sludge</td>
<td>Batch MBR with total recycle mode [15 Lmh]</td>
<td>Maximum TMP 36–39 kPa [76–79 kPa in control] in 3 operation cycles (15–20 h) TMP 10 kPa throughout the experiment [30 kPa in 48 h for the control]</td>
<td>Yeon et al. (2009b)</td>
</tr>
<tr>
<td>QQ</td>
<td>Microbial vessel containing recombinant E. coli</td>
<td>Continuous MBR [20 Lmh]</td>
<td>39 h to reach 25 kPa [28 h in control]</td>
<td>Oh et al. (2012)</td>
</tr>
</tbody>
</table>

a QQ: Quorum quenching; ED: Enzymatic disruption.
b TMP: trans-membrane pressure.
photometric methods or TOC measurements, only surrogate concentrations are determined and no information on individual constituents or synergistic effects is obtained so that two samples with equal net carbohydrate, protein or TOC concentration can exhibit largely different behaviors (Drews, 2010). Measurement of mechanical properties in EPS such as elastic and shear modulus, adhesive strength or tensile strength can be challenging but a range of values for these properties is reported in the literature (Yun et al., 2006; Gao et al., 2011a; Sweity et al., 2011).

### 3.1.2. Implications from EPS studies

Contradictory results in the literature are commonly found when addressing the influence of a specific parameter on membrane biofouling, especially those related to EPS. For example, in some MBRs, protein was identified as the main foulant (e.g. Ng and Ng, 2010; Gao et al., 2011b; Wang et al., 2011) and thought to be more involved than sugars in generating electrostatic bonds with multivalent cations, a principal factor in stabilizing aggregate structures (Dignac et al., 1998). Carbohydrates were nonetheless found to show a better correlation to membrane permeability in other studies (Germain et al., 2005; Kimura et al., 2005). Using NMR and FTIR, Kimura et al. (2005) found that with higher food-microorganisms ratio (F/M), membrane foulants became more proteinaceous. Metzger et al. (2007) fractionated MBR fouling layers by different removal mechanisms and identified an upper loosely bound layer with a similar composition to the biomass flocs, an intermediate fraction with a higher concentration of carbohydrates and a lower layer featuring a relatively higher concentration of strongly bound proteins. The generation of EPS in the lower fraction of the bio-cake layer over long periods of operation was also found to be responsible for the rapid increase in TMP under subcritical flux (Hwang et al., 2008). Loosely-bound EPS was found to show more significant positive correlation with fouling than tightly-bound EPS during long term operation of a pilot-scale MBR (Wang et al., 2009).

It is the general current consensus that proteins and carbohydrates are assumed to be the major fractions contributing to fouling, although other components may also play a significant role. For example, Subhi et al. (2013) found that biopolymers and low-molecular-weight neutrals were the major contributors to irreversible fouling. During studies of alginate biosynthesis in P. aeruginosa, Whitchurch et al. (2002) discovered that the majority of the extracellular material was not exopolysaccharides but DNA and found that addition of DNase I to the culture medium strongly inhibited biofilm formation. Lyko et al. (2007) found an important influence on MBR membrane fouling by both soluble humic substances and carbohydrates in complexes with metal cations. Three-dimensional excitation-emission matrix (EEM) fluorescence spectra analysis also revealed that membrane fouling was associated with fulvic acid-like substances (Wang et al., 2011). These findings highlight the critical role of the method of analysis chosen and that can only measure a limited amount of compounds (Subhi et al., 2011). The above contradictions also emphasize the need for standardization in measurement methods and analytical tools and for well-controlled experiments. Proper measurement of trans-membrane pressure and early biofouling detection are also important in MBRs as has been shown in other types of membranes (Vrouwenvelder et al., 2009a, 2011). Future studies employing more of the advanced techniques can reveal information on EPS fingerprints and origins beyond structure and composition. However, protocol optimization and adequate sample purification for new techniques such as MALDI/TOF/MS are important (Kimura et al., 2012). Furthermore, in lab-scale MBRs, operating and environmental conditions are subject to higher fluctuations than in large plants and time scales often differ significantly, which stresses the importance of conducting more studies on real-scale MBRs in order to validate laboratory research findings.

### 3.2. Advanced microbial analysis of MBR fouling

In addition to EPS studies, it is important to properly characterize the microorganisms that are responsible for biofouling. This provides the basis for endeavors directed at developing biological-based antifouling strategies. With the advancement of molecular biology techniques, more studies have been conducted to characterize microbial communities responsible for biofouling in MBRs. Table 3 gives a brief description of the analytical tools used for characterizing both the microbial communities (structure and function) and biofilms (microorganisms with associated EPS matrix) on membrane surfaces.

#### 3.2.1. Analytical tools for microbial community characterization

Culture-based microbial characterization approaches introduce well-known biases such as the potential to exclude the detection of important microbial species (Herzberg et al., 2010). These approaches tend to select for the fittest and least fastidious microorganisms, whereas non-culture-based molecular biology techniques can provide more reliable and useful information (Table 3). Pre-genomic tools refer to DNA or RNA-based molecular techniques that target a single gene (commonly the 16S rRNA gene) or RNA molecule. These tools are important for assessing microbial community structure and identifying functionally important members of uncharacterized communities (Rittmann et al., 2008). However, the obtained information on community structure and diversity is only weakly linked to metabolic and functional capabilities (Tringe and Hugenholtz, 2008).

With the advent of genomic (metagenomic) and post-genomics (metatranscriptomic and metaproteomic) approaches, as described in Table 3, methodological studies of expressed subsets of genes or proteins can be conducted. Metagenomics can unveil a large number of sequence reads with unidentified origin, offering information on microbial diversity, gene content and metabolic potential (Taylor et al., 2007; Warneckea, and Hess, 2009; Rademacher et al., 2012). Post-genomic approaches such as metatranscriptomics and metaproteomics give information on community function by identifying the messenger RNA (expressed gene transcripts) or expressed proteins and can reveal the link between community structure and function (Wilmes and Bond, 2006; Helbling et al., 2011). These high throughput next-generation sequencing techniques surpass conventional methods in
### Table 3 – Available analytical tools for membrane biofouling characterization.

<table>
<thead>
<tr>
<th>Technique*</th>
<th>Principles</th>
<th>Information gained &amp; limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular biology techniques</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-genomic</td>
<td>DGGE</td>
<td>Amplified gene fragments are separated on a denaturing gel based on their different melting behavior</td>
</tr>
<tr>
<td></td>
<td>Clone library</td>
<td>PCR amplified gene fragments are inserted into a plasmid and sequenced using Sanger sequencing</td>
</tr>
<tr>
<td></td>
<td>T-RFLP</td>
<td>PCR amplified fluorescently labeled terminal restriction fragments (T-RFs) are separated based on size or capillary electrophoresis &amp; identified with automatic DNA sequencer</td>
</tr>
<tr>
<td></td>
<td>16S rRNA gene pyro-sequencing</td>
<td>A high-throughput sequencing technology capable of obtaining few thousand sequences of partial 16S rRNA gene; using barcoded primers, hundreds of PCR amplified samples (amplicons) can be sequenced simultaneously</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>Fluorescently labeled DNA oligonucleotide probes hybridize to RNA molecules (rRNA or mRNA)</td>
</tr>
<tr>
<td><strong>Genomic</strong></td>
<td>Meta-genomics</td>
<td>Relies on shotgun sequencing, isolated DNA are fragmented, cloned into a suitable vector and sequenced; with massive parallel sequencing platforms (Roche 454, Illumina, ABI SOLiD) generates large number of short sequence reads</td>
</tr>
<tr>
<td><strong>Post-genomic</strong></td>
<td>Meta-transcriptomics</td>
<td>Isolated RNA are sequenced using pyrosequencing technology (Roche 454), which is capable of obtaining ten to hundreds of thousands sequences from the RNA pool</td>
</tr>
<tr>
<td></td>
<td>Meta-Proteomics</td>
<td>Extracted proteins are separated on a gel followed by mass spectrometry or are digested into peptides followed by separation and identification using liquid chromatography tandem mass spectrometry (LC-MS/MS)</td>
</tr>
<tr>
<td><strong>Biofilm characterization methods</strong></td>
<td>Ultrasonic reflectometry</td>
<td>The reflected time and amplitude of sound waves are compiled into frequency distributions</td>
</tr>
<tr>
<td></td>
<td>CLSM</td>
<td>Uses white lasers for providing 3D information of the biofouling layers and studying the overall biofilm morphology with high resolution</td>
</tr>
<tr>
<td></td>
<td>MRI</td>
<td>Non-invasive characterization using magnetic field gradients to obtain nuclear spin density images</td>
</tr>
<tr>
<td></td>
<td>STXM</td>
<td>Provides differentiation of classes of biomolecules without the use of fluorescent labels; allows chemical speciation &amp; compositional mapping based on bonding structure</td>
</tr>
<tr>
<td></td>
<td>SIMS + FISH</td>
<td>FISH probes are imaged using SIMS so that several elements can be observed simultaneously</td>
</tr>
<tr>
<td></td>
<td>AFM</td>
<td>Mapping of the distribution of macromolecules</td>
</tr>
<tr>
<td></td>
<td>SEM/TEM</td>
<td>Magnified imaging of biofouling layers using electron beam</td>
</tr>
</tbody>
</table>

---

*DGGE, denaturing gradient gel electrophoresis; T-RFLP, terminal restriction fragment length polymorphism; FISH, fluorescent in situ hybridization; rRNA, ribosomal RNA; mRNA, messenger RNA; CLSM, confocal laser scanning microscopy; MRI, magnetic resonance imaging; STXM, scanning transmission X-ray microscopy; SIMS, secondary-ion mass spectrometry; AFM, atomic force microscopy; TEM, transmission electron microscopy; SEM, scanning electron microscopy.
profiling complex bacterial communities (Kwon et al., 2010; Ye and Zhang, 2011; Guo and Zhang, 2012); however, the enormous amount of generated data requires proper bioinformatic analysis, involving the assembly, mapping and interpretation of large quantities of short sequence reads (Simon and Daniel, 2009; Horner et al., 2009; Vakhlu et al., 2012). Another challenge is associated with the difficulty to convert this data into practical solutions and/or better understanding of the biofouling phenomenon. The application of genomic and post-genomics methods are still limited in the field of biofouling in membranes for water/wastewater treatment in general, or in MBRs in particular.

Biofilm characterization techniques and image-analysis algorithms offer growing opportunities to better understand the biofouling process. For example, atomic force microscopy (AFM) can distinguish variations in cell deposition (Zaky et al., 2012). CLSM images help determine time-dependent development of microcolonies on membrane surfaces (Miura et al., 2007; Zator et al., 2007). Other biofilm characterization methods (Table 3) are also available but have not been used to their full potential in MBR studies. In reverse osmosis (RO) modules, techniques such as magnetic resonance imaging (MRI) have been applied to capture water flow and subsequent changes in biofilm distribution due to cleaning (Creber et al., 2010). Vrouwenvelder et al. (2009b) employed in-situ non-destructive MRI to support visual observations of pressure drop measurements in spiral-wound NF and RO membranes. In MBRs, the application of these advanced biofilm characterization methods is still limited. The main limitation is the higher complexity of MBR systems and the need to prepare well-designed experimental setups with specific and methodical objectives. Accordingly, a broad scope for extensive research exists in this area. A systematic approach that integrates emerging 'omics' technologies and combinations of biofilm characterization methods offers a wealth of opportunities to collect multiple pieces of information on biofilm development on membrane surfaces. The proper selection of these tools is vital for ultimately developing better biological anti fouling strategies that can also benefit the advancement of physical, chemical as well as other non-biological control methods.

3.2.2. Biofouling microbial communities in MBRs

Many studies reported the phylum Proteobacteria to be the dominant phyla in biofouled MBR membranes (Lim et al., 2004; Choi et al., 2006; Jinhua et al., 2006; Miura et al., 2007; Zhang et al., 2006; Ahmed et al., 2007; Huang et al., 2008b; Duan et al., 2009; Fontanos et al., 2010; Gao et al., 2011b; Piasecka et al., 2012). In addition to Proteobacteria, other phyla such as Firmicutes (Lim et al., 2004; Calderon et al., 2011), Bacteroidetes (Fontanos et al., 2010; Huang et al., 2008b), Actinobacteria (Lim et al., 2004) and Planctomycetes (Piasecka et al., 2012) have also been reported to be predominant in MBR fouled membranes. Some researchers argue that targeting pioneer bacteria that initially colonize on membrane surfaces might help develop better control strategies; yet, very few studies have addressed pioneer bacteria (Zhang et al., 2006; Piasecka et al., 2012). Choi et al. (2006) noted that Acinetobacter is important in the early colonization of MF membrane in a lab-scale MBR. Miura et al. (2007) noted that initially the biofilm in hollow-fiber MF membrane of MBR treating municipal wastewater was composed of Alpha-, Beta-, Gamma- and Delta-Proteobacteria whereas in mature biofilm, Dechloromonas-related bacteria became dominant. Zhang et al. (2006) reported a list of bacteria that might be the pioneer bacteria colonizing the surface of flat-sheet MF membranes in an MBR treating synthetic paper mill wastewater. Most of the sequences belonged to Alpha-, Beta- and Gamma-Proteobacteria, the Cytophaga-Flavobacterium-Bacteroides clade, and an uncultured bacterium division TM7. Gao et al. (2011b) reported that species belonging to the phylum Alphaproteobacteria were prone to form initial biockate but as conditions become more anoxic in thicker biockate, habitats were changed with a succession from Betaproteobacteria to Deltaproteobacteria. Piasecka et al. (2012) identified 25 pioneer operational taxonomic units (OTUs) belonging to the phyla Firmicutes, Proteobacteria, Bacteroidetes and Planctomycetes on membrane surface after one day of filtration in a lab-scale MBR with double-flat sheet membranes operated on molasses wastewater. In an inclined plate MBR, Fontanos et al. (2010) reported that although the same biofouling microbial groups were found on polyethylene (PE) and polyvinylidene fluoride (PVDF) hollow fiber membranes, the community dominance exhibited different trends with the advent of fouling.

3.2.3. Implications in MBRs and other membrane applications

A common finding in several studies is that the microbial community identified on biofouled MBR membranes differs significantly from the mixed liquor community (Piasecka et al., 2012; Gao et al., 2010; Huang et al., 2008b; Miura et al., 2007; Zhang et al., 2004; Choi et al., 2006; Jinhua et al., 2006). For example, Miura et al. (2007) noted that the filamentous Chloroflexi, which accounted for nearly 20% of total hybridized cells in the mixed liquor was not detected on the membrane surfaces of pilot-scale MBRs. Similarly, Gao et al. (2010) noted the absence of Bacteroidetes and other bacteria that were abundant in suspended biomass. Choi et al. (2006) and Zhang et al. (2004) further reported that genera such as Brevundimonas, Acinetobacter, Sphingomonas, and Aquaspirillum, which were present in low abundance in the mixed liquor became dominant on fouled MF membranes, implying that specific bacterial populations selectively attached to the membrane surface. Piasecka et al. (2012) observed that this difference between mixed liquor and biofouling communities was more pronounced in the initial phases of filtration.

Research in process water RO systems has been successful in identifying a specialized bacterial responsible for initiating and dominating biofouling i.e. Sphingomonas spp. (Bereschenko et al., 2010). A common cosmopolitan group of biofouling bacteria was, moreover, identified on five different seawater RO membranes from different parts of the world, operated under different conditions and collected at different times of the year (Zhang et al., 2011). Whether specific initiating and dominating species can be identified in MBRs and if so, how this information can be linked to developing better control needs to be determined by first filling specific knowledge gaps that still remain in MBRs.
4. Current gaps in knowledge

4.1. Microbiological studies

Although the genetic basis of biofilm formation has been investigated for several bacterial species, including E. coli (Pratt and Kolter, 1998), P. aeruginosa (Heydorn et al., 2000; O’Toole and Kolter, 1998) and Vibrio cholera (Watnick and Kolter, 1999), similar studies targeting the specific microorganisms responsible for membrane biofouling in MBRs are very limited. Successful application of biological-based antifouling strategies requires a deeper understanding of the microbial ecology of the communities responsible for biofouling and which may completely differ from the ones that have been investigated in the literature on biological control in biofilms. Despite the increasing number of studies, the microbial ecology of microorganisms responsible for biofouling in MBRs has not been systematically addressed. Whereas some researchers claim that targeting the pioneer bacteria is important (Zhang et al., 2006; Piasecka et al., 2012), severe fouling is often observed in initial phases when the biomass is not yet fully acclimated (Li et al., 2012), most existing microbial studies in MBRs have focused on the mature bacterial biofilm ecology (Lim et al., 2004; Jinhua et al., 2006; Miura et al., 2007; Huang et al., 2008a; Duan et al., 2009; Gao et al., 2010).

On the other hand, only one study has characterized archaea in biofouling layers where in addition to the dominant bacterial phyla (Firmicutes and Alphaproteobacteria in this case), archaenal clones – mostly affiliated to the order Methanosarcinales and family Methanospirillaceae were identified (Calderon et al., 2011). Few studies in the literature were based on real wastewater (e.g. Huang et al., 2008b; Miura et al., 2007; Jinhua et al., 2006; Lim et al., 2004) while most others used synthetic wastewater and the majority of microbiological studies were conducted on lab-scale MBRs with very few on pilot-scale systems (Calderon et al., 2011; Jinhua et al., 2006) and none on real-scale systems. Moreover, existing microbiological studies in MBRs have focused only on the microbial community structure (who is there?) but not function (i.e. expressed genes and proteins). Studying both the structure and function of the biofouling community is important in future microbiological studies since a common function may be identified for different microbial communities. Common genes may be identified for important functions (e.g. EPS formation or QS) even from different phylogenic groups. Advanced biofilm characterization and next-generation high throughput sequencing tools (metagenomics, metatranscriptomics) can be especially helpful in this regard as illustrated in Table 3. So far, existing microbiological studies in MBRs have utilized tools such as T-RFLP and clone library analysis of 16S rRNA gene (Piasecka et al., 2012; Gao et al., 2010), DGGE and clone library analysis of 16S rRNA gene (Duan et al., 2009; Huang et al., 2008b; Jinhua et al., 2006), quinone profile analysis (Ahmed et al., 2007; Lim et al., 2004), amplified ribosomal deoxyribonucleic acid restriction analysis (ARDRA) and clone library analysis of 16S rRNA gene (Zhang et al., 2006) and FISH (Fontanos et al., 2010; Miura et al., 2007).

Genomics and post-genomics techniques are expected to offer new clues to better control, but how this information can be channelized into devising effective, sustainable and cost-effective control strategies still needs further research. Although technical challenges such as DNA, RNA, and protein extraction, mRNA instability and lack of reference genomes may hamper the use of omics for some time, the growing use of these techniques offers the opportunity for a more comprehensive understanding of the structure and function of biofouling communities. Post-genomic approaches have been successfully applied to detect expression profiles (mRNA and proteins) and provide functional insights for mixed culture environmental samples such as the gut microbiota (Gosalbes et al., 2011) and activated sludge (Wilmes et al., 2008). One successful story of applying these tools is in enhanced biological phosphorous removal (EBPR) processes. Combining new sequencing technologies from -omics approaches with single-cell studies and with process and physical/chemical studies allowed linking intracellular metabolite flux models to comprehensive system-scale models for EBPR (Nielsen et al., 2012). In the field of membrane biofouling, -omic tools are still not widely applied. Miyoshi et al. (2012) analyzed two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) spots by N-terminal amino acid sequencing analysis and identified two well-characterized outer membrane proteins originating from Pseudomonas genus, namely OprF and OprD, contributing to membrane fouling in MBRs. More studies employing these tools can play a vital role in clarifying the functions and interactions among biofouling microorganisms and could help improve our understanding of critical aspects of the biofouling process. The practical success with filamentous bacteria is a good demonstration of manipulation of populations for better control of these microorganisms. Molecular tools have been applied to study filamentous bacteria, which can cause poor settling (bulking) or foaming. Because of an increased understanding of their identity and ecology, it has been possible to develop specific control measures for several of these unwanted organisms, which include changes in plant operation, process layout or addition of chemicals (Nielsen et al., 2009). Thus, a better understanding of the microbiology and proper linking with operational parameters is expected to help in developing more effective control strategies and cleaning methods.

4.2. Biological strategies for MBR antifouling

4.2.1. Challenges in biological-based strategies

Most of the biological-based antifouling strategies are still in the developing phase and many face their own challenges and limitations. In QQ, for example, care should be taken so that desirable biochemical reactions undertaken by the microbial consortia would not be affected by a particular quorum-quenching strategy (Choudhary and Schmidt-Dannert, 2010; Jiang et al., 2013). Inhibition of ATP synthesis by chemical uncouplers may be a feasible alternative for alleviating biofouling, but it has the disadvantage that most ATP uncouplers are classified as aromatic and can be toxic (Xiong and Liu, 2010), thereby limiting their practical application. Conversely, the enzymatic method is nontoxic and environmentally friendly but enzymes are unstable and are highly
pH-, temperature-, and salt concentration-sensitive. For bacteriophages, in addition to phage resistance challenges, nutritional limitation is known to influence the stability of bacteria—phage interactions where increasing the input of nutrients can lead to a large increase in phage numbers, a small increase in bacteria and a reduction in the dynamic stability of both populations (Bohannan and Lenski, 1997). Also, the presence of a non-susceptible bacterial population could protect bacteriophage-susceptible strains, possibly by creating ‘spatial refugees’ within the depths of the biofilm (Tait et al., 2002).

Despite the success of recent studies described before (e.g. QQ used by Oh et al., 2012), more research is needed to examine the scale-up of these results to real MBRs and validate their effectiveness using real wastewater. Much remains to be learned about the role of QS in biofilm formation. For instance, more than 100 species of Proteobacteria, which is a common phylum of biofouling bacteria, are known to contain AHL signal genes, which could be responsible for QS signaling pathways; however, only few of the species identified in biofouled membranes show QS-regulated control of EPS regulation and many do not have clear functions associated with their QS circuitry (Shrout and Nerenberg, 2012). Moreover, many microorganisms possess QS genes but not all of these genes are expressed. Different microorganisms also generate different QSs. Therefore, for QQ-based strategies to hold more promise in biofouling management, it is important to learn more about the eco-physiology of biofouling microorganisms.

The practicality, specific design and operational requirements as well as the economic feasibility of expanding lab-scale experiments to real-scale systems still need deeper assessment and more research on real-scale applications. Also, most of the well-studied systems in biological control studies have been studied for pure cultures while uncovering the details for mixed microbial cultures and other species such as Gram-positive bacteria, archaea and fungi is needed (Choudhary and Schmidt-Dannert, 2010; Calderon et al., 2011). Through proper application of high throughput analytical tools, the specific microorganisms and genes responsible for MBR biofouling can be identified. This information can be used in developing targeted and cost-effective biological control approaches in contrast to the present status of disjointed and still rudimentary trials followed in biological control approaches. Consequently, studies on the effectiveness of biofilm dispersal and inhibition techniques still need to be broadened to validate their applicability to specific operational conditions in MBRs and prove their effectiveness on the key microbial species responsible for MBR biofouling.

Effective biofilm control practices are expected to originate from well-selected and directed strategy combinations. A single approach must be 100% effective, whereas in combination each individual approach can be partially effective while the combination is still efficient (Vrouwenvelder et al., 2010). As evidenced in several laboratory studies, any one process modification alone is unlikely to solve all accumulation of undesired biofilm growth (Shrout and Nerenberg, 2012). For example, a furanone inhibitor of P. aeruginosa QS increased the efficacy of detergents or antibiotics in biofilm killing (Hentzer et al., 2003) and exogenous addition of autoinducer peptide (AIP) increased the sensitivity of biofilms to antibiotics (Lauderdale et al., 2010). Combinations of phage enzymes and disinfectants by adding the phage and then the disinfectant have also been found to be more effective than adding either alone (Tait et al., 2002). Similarly, Hughes et al. (1998) demonstrated that phage polysaccharide depolymerases afforded better access for disinfection and better EPS removal. Chlorine treatment was also found to be 20 times more effective at removing multispecies biofilms from water systems after exposure to nitric oxide, which can disrupt the intracellular secondary messenger cyclic di-GMP in microorganisms (Barraud et al., 2009). Another agent that disrupts intracellular signaling, not necessarily through QS pathways, is cis-2-decanolic acid, which acts as a fatty acid messenger that can induce biofilm dispersion (Davies and Marques, 2009). Both nitric oxide and cis-2-decanolic acid seem to be appealing due to their multispecies efficacy, natural synthesis, and readily biodegradable characteristics (Shrout and Nerenberg, 2012); however, their incorporation to cleaning protocols in real-scale systems and in properly designed and cost-effective strategies remains a challenge.

Gino et al. (2010) proved the potential of combined chemical-biological treatment in preventing groundwater well clogging as a result of biotic iron oxidation. Glycolic acid (2%) and isolated bacteriophages showed inhibition of biofilm formation while earlier biofilm treatment with reduced glycolic acid concentration revealed efficient EPS digestion thus allowing phages to be more efficient against biofilm matrix bacteria. The above examples show that combined approaches might offer a greater potential for effectively limiting biofouling problems than a single control method. The appropriate doses, designs and operational aspects to be applied in real systems still need to be determined. Newly developed approaches should also not interfere with the biodegrading activities and desired functions of microorganisms in the mixed liquor. For instance, Meng et al. (2012) reported that ciprofloxacin could inhibit filaments in MBR mixed liquor but with an adverse effect on denitrification.

In addition to integrating effective biological-based strategies with optimized cleaning methods, a combined systematic approach that links microbiology with MBR design and operational aspects is essential to understand how these parameters are interrelated and to effectively manage biofouling for the wide range of conditions governing MBR operation.

4.3. Linking operation to microbiology

In MBRs, many factors, including reactor design and operation, cleaning strategies, membrane properties and biomass conditions can have direct impact on MBR fouling potential (Yamato et al., 2006; Meng et al., 2009). Kraume et al. (2009) noted that differently sized modules, lack of appropriate hydrostatic head, presence of aerators, differing backflush or relaxation hours might result in different fouling mechanisms. These parameters are also expected to impact the microbial community structure; yet, systematic correlations that link the biofouling community structure and function to important operational and environmental parameters are still
Miura et al. (2007) found that with higher aeration rates, beta-proteobacteria became the most dominant biofouling species. Lim et al. (2004) reported the presence of Paracoccus sp. and Flavobacterium sp. for continually aerated and Pseudomonas, Moraxella, Vibrio, Staphylococcus warneri, Micrococcus sp. and Nocardia sp. for intermittently aerated hollow fiber MF membranes in MBRs. Ahmed et al. (2007) reported the growth of microorganisms from the delta- and epsilon-subclass of Proteobacteria and members of the Cytophaga–Flavobacterium cluster increased at longer sludge retention times (SRT) whereas in the study by Duan et al. (2009), beta-proteobacteria dominated at all SRTs. Huang et al. (2008a) found that at lower fluxes, the MBR biofilm community composition was similar independent of sludge age whereas distinct biofilm communities developed on membrane surfaces at high fluxes. Despite the significance of these research findings, more studies on other parameters are still needed particularly relating to reactor design, operation and applied cleaning protocols and how these impact biofouling communities.

Similarly, the amount and temporal sequence of EPS formation in response to different physical and biological conditions are unknown and the potential to predict EPS production is the weakest point in EPS research (Flemming and Wingender, 2010). In general, EPS composition, amount and properties are likely to be unique for each species but very little is known about EPS production for most fouling species (Shrout and Nerenberg, 2012). Using the emerging advanced molecular and imaging tools, it may be possible to study the particular genes responsible for biofouling at each stage of biofilm development e.g. initial attachment, growth, EPS secretion and detachment and how the expression of these genes varies for different conditions.

When characterizing the microbial communities and their associated products in MBRs, it is important to consider: a) the use of real wastewater compared to synthetic wastewater. A lower microbial diversity index is generally attributed to systems with synthetic influent regardless of operation mode and reactor scale (Hiraishi et al., 1998); b) long-term monitoring of the biofouling communities since deviations in operational conditions and in membrane properties, as foulants attach to the surface, can have a significant impact on the bacterial eco-physiology. It has been reported that short-term membrane adhesion tests are not a suitable predictor for biofouling in MF and UF membranes, which showed significantly reduced adhesion of biofoulants in short-term tests, but no reduction during longer biofouling experiments (Miller et al., 2012); and c) influence of recurrent cleaning and the relationship between the biofouling bacterial communities and the cleaning option adopted. Fig. 1 depicts the interacting variables as well as the possible integration of relevant analytical tools for developing a better understanding of MBR biofouling.

5. Conclusion

Membrane biofouling is an inevitable problem that has become an intensive research area, demanding a methodical
and unified investigation approach. In practice, a more effective integrated control approach should be based on three considerations: system design and operation, biomass growth conditions and cleaning strategies. The key argument presented here to pursue microbiological routes as an essential ingredient in an integrated control of biofouling stems from their high effectiveness potential, sustainability and possibly lower costs. Biological methods offer a promising control alternative but more investigations on the structure and function of biofouling bacterial populations and their EPS are needed. Also, future studies should specifically address the relationship between the biofilm communities and environmental, operational (including cleaning) and membrane conditions, as well as the application of successful biofilm control methods to MBRs.

Acknowledgments

The preparation of this article was supported by discretionary investigator funds (Pascal Saikaly) at King Abdullah University of Science and Technology (KAUST).

REFERENCES


Gao, D., Fu, Y., Tao, Y., Li, X., Xing, M., Gao, X., Ren, N., 2011b. Linking microbial community structure to membrane
biofouling associated with varying dissolved oxygen concentrations. Bioresource Technology 102, 5626–5633.


