Influences and impacts of biofouling in SWRO desalination plants.

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Abstract

The ability to produce fresh potable water is an ever-growing challenge, especially with an increase in drought conditions worldwide. Due to its capacity to treat different types of water, reverse osmosis (RO) technology is an increasingly popular solution to the water shortage problem. The major restriction associated with the treatment of water by RO technology is the fouling of the RO membrane, in particular through biofouling. Membrane fouling is a multifaceted problem that causes an increase in operating pressure, frequent cleaning and limited membrane lifespan. The current paper summarizes the impact of biofouling of RO membranes used in seawater desalination plants. Following a brief introduction, the elements that contribute to biofouling are discussed: biofilm formation, role of extracellular polymeric substances (EPS), marine environment, developmental phases of biofouling. Following this, is a section on the implications of membrane biofouling especially permeate flux and salt rejection. The final section focuses on the new phenomenon of compression and hydraulic resistance of biofilms. Lastly, considerations on future research requirements on biofouling and its control in seawater reverse osmosis (SWRO) membrane systems are presented at the end of the article.

Keywords: Biofilm; Hydraulic resistance; Permeate flux decline; Salt rejection; Aggregates; Transparent exopolymer particles (TEP).

Introduction

Water scarcity is a growing problem worldwide, with the demand escalating due to increasing population, uneven water distribution and rigorous quality regulations. Future water shortages are being recognised as a significant problem as much of the global economy relies upon sustainable high-quality water (Lee and Kim, 2011, Matin, Khan, Zaidi, Boyce, 2011). A vast majority of the water that is accessible for direct consumption is not consumable due to its salinity level; therefore, developing alternative technologies in an effort to provide water resources is a continual challenge. A commonly used clean water technology is RO membrane desalination (Goh, Matsuura, Ismail, Ng, 2017).

SWRO desalination is considered to be one of the most cost-effective methods of producing potable water. The desalination process involves the feeding of water under high pressure across a semi permeable membrane to reject salts, organic and biological matter including bacteria and viruses and obtaining fresh water (Lee & Kim, 2011, Matin *et al*., 2011).

RO is a membrane-based pressure driven system wherein the membrane separates unwanted components from the feed water to obtain a pure product. SWRO desalination uses a semi permeable membrane to reject salt while allowing the selective transport of water through to produce clean water. Worldwide reliance on RO is increasing due to its versatility and continuous technological improvements that have resulted in cost reductions and increased energy efficiency (Harif *et al.*, 2011, Lee and Kim, 2011, Goh et al., 2017). In particular, RO allows for the removal of large amounts of dissolved solids, organics, colloidal matter and microorganisms via a semi permeable membrane (Lee & Kim, 2011, Matin *et al*., 2011). Due to the high rejection feature and the efficiency of the membranes, SWRO is considered to be the simplest and most cost effective method of freshwater production in comparison to other separation methods such as

distillation, solvent extraction, ion exchange and adsorption (Lee & Kim, 2011, Matin et al., 2011, Farahbakhsh, Delnavaz, Vatanpour, 2017, Goh et al., 2017).

While RO technology has many applications, membrane fouling is a significant shortcoming that limits the efficiency of the RO process. Even after seawater pre-treatment and cross flowing within the RO system, aquatic organisms and organic compounds still enter the RO module and the dynamic process of colonisation and growth on the membrane causing biological fouling (Flemming, 2002, Ivnitsky *et al*., 2007, Komlenic, 2010, Kochkodan & Sharma, 2012). There are many different types of fouling such as inorganic fouling, particles/colloids, organic fouling and biofouling (Flemming, 1997, Reverter, Talo & Alday, 2001, Fujiwara & Matsuyama, 2008, Bartman, Lyster, Rallo, Christofides & Cohen, 2011).

Fouling is the reversible or irreversible attachment of organic or inorganic particles to the surface of the membrane (Hong and Elimelech, 1997, Belfer, Purinson, Fainshtein, Radchenko, Kedem, 1998). Several factors including intake water quality and pre-treatment measures drive RO membrane fouling. Pre-treatment barriers such as micro-filtration (MF) and ultra-filtration are commonly used in SWRO desalination plants to reduce the amount of foulants reaching the RO membrane, inducing biofoulants (Rapenne, Port, Roddy, Croué, 2007, Mo, Tay, Ng, 2008). Biofilm formation on the RO membrane creates adverse effects on the operation of desalination plant, resulting in a decline in salt rejection, water quality and flux and an increase in operating pressure (Herzberg, Kang, Elimelech, 2009, Berman, Mizrahi, Dosoretz, 2011, Harif *et al*., 2011).

This paper aims at summarizing the globally significant topic of biofouling of membranes within saltwater reverse osmosis desalination plants based on existing literature. The review starts with the formation of biofilms and elements that contribute

to the biofouling of RO membranes. Subsequently, the developmental phases of biofouling are discussed with a focus on a new paradigm. The implications that biofouling has on membranes, especially permeate flux and salt rejection, are also reviewed. Next, a new phenomenon will be examined, the compression and hydraulic resistance in biofilms. Finally, we reflect on the differences between biofilms formed naturally and under pressure before presenting our considerations on future research requirements on biofouling and its control in SWRO membrane systems.

Elements contributing to the biofouling of reverse osmosis membranes

Within pelagic ecosystems, key processes such as flux, cycling and sedimentation of elements and energy have been extensively studied over the years. Rich in populations, these plankton organisms consist of bacteria, archaea, algae, protozoa and multicellular zooplankton (Hays, Richardson, Robinson, 2005). Most oceanic pelagic systems are nutrient poor, stratified systems in which picoplankton are the dominant component of the planktonic biomass (Berglund, Müren, Båmstedt, Anderssonm, 2007). Prokaryotes make up a major component of the picoplankton biomass in marine environments and are an integral component of the microbial food web (Sherr and Sherr, 1988, Kiørboe *et al*., 1990, Sommer, Stibor, Katechakis, Sommer, Hansen, 2002). This microbial loop is an essential link between dissolved organic matter (DOM) and the higher trophic levels and is made available when metabolised by bacteria. Therefore, the role of microbes within the pelagic region is to regulate the energy flow through the foodweb, thereby, limiting the export of biomass towards the benthos.

Marine microbes have the specificity to produce extracellular polysaccharides in the form of transparent exopolymer particles (TEP) that have been found to be species specific and dependent on surrounding environmental (growth) conditions (Gordon, 1970, Alldredge, Passow & Logan, 1993, Passow and Alldredge, 1994, Simon, Grossart, Schweitzer & Ploug, 2002, Verdugo *et al*., 2004, Li *et al*., 2016, Taucher *et al*., 2018). TEP are deformable, gel like transparent particles that appear in many forms, such as amorphous blobs, clouds, sheets, filaments or clumps (Chin, Orellana & Verdugo, 1998, Verdugo *et al*., 2004, Linares, Yangali-Quintanilla, Li & Amy, 2012). In the marine environment, they have been found to range in size from micro- up to milli-metre scales, but can also span several centimetres through the formation of dense networks (Azetsu-Scott & Passow, 2004). These particles can be formed spontaneously from the aggregation/encounter of dissolved precursor substances, as well as by the type and concentration of precursors present in the water (Passow, 2002, Thuy et al., 2015). TEP can also be produced from colloidal material through the breakdown of algal aggregates by bacteria (Kiørboe & Hansen, 1993, Hansen, Timm & Kiørboe, 1995, Passow & Alldredge, 1995, Grossart, Simon & Logan, 1997, Mari & Burd, 1998, Engel, 2000). TEP, which consist of EPS with the addition of high surface-active polymers, are likely to have a role in coating surfaces and providing a nutritious substrate for bacteria and other microorganisms to colonise (Bar-Zeev, Berman-Frank, Girshevitz & Berman, 2012). In the marine environment, TEP serve as “hot spots” of intense microbial and chemical activity within the water column facilitating the attachment of planktonic TEP to surfaces (Berman, Mizrahi & Dosoretz, 2011). When attached to surfaces such as biofilms or macroaggregates, marine microbes have the capability to produce exopolysacchrides in large amounts (Decho, 1990, Costerton, Lewandowski, Caldwell, Korber & Lappinscott, 1995, Heissenberger,

Leppard & Herndl, 1996, Stoderegger & Herndl, 1998). However, when non-aggregated in the water column, they are also reported to produce TEP.

Those aggregates formed by microorganisms in the pelagic realm are commonly called marine snow. Marine snow is regarded as aggregates, of 0.5 mm or larger in diameter, which are highly diverse in origin, morphology and characteristics within marine environments (Alldredge & Silver, 1988, Silver, Coale, Pilskaln & Steinberg, 1998, Simon *et al*., 2002, Burd & Jackson, 2009, Iversen & Ploug, 2010). The structural components of aggregates therefore vary from fragile, porous, loosely associated smaller particles and organisms to those that are extremely cohesive, robust and gelatinous in structure (Alldredge & Silver, 1988, Taucher *et al*., 2018). Marine snow is primarily formed from algae, inorganic particles, zooplankton feeding structures, faecal pellets and detritus (Alldredge & Gotschalk, 1988, Alldredge & Gotschalk, 1990, Hansen *et al*., 1996, Alldredge, Passow & Haddock, 1998). The formation of marine snow via physical aggregation is enhanced through two biological-mediated pathways (Alldredge & Silver, 1988). First, via the production of sticky mucus, exopolymers or products of cell lysis which increase the probability of colliding particles attaching, and also through the probability for potential collision resulting from biological alteration of the size and surface characteristics of the particles (Alldredge & Silver, 1988, Jackson, 1990, Riebesell, 1991, Burd, 2013, Taucher *et al*., 2018). Marine snow can be seen as macromolecular structures containing bacterial biofilms associated with the suspended particles (Gupta, Sarkar, Das, Bhattacharjee & Tribedi, 2016).They frequently contain higher concentrations of organic and inorganic particles than that of the surrounding environment (Shanks & Trent, 1979, Prézelin & Alldredge, 1983, Kiørboe & Jackson, 2001, Grossart, Hietanen & Ploug, 2003), often resulting in heavy colonisation by heterotrophic bacteria (Alldredge & Youngbluth, 1985, Alldredge, Cole

166 & Caron, 1986, Simon *et al*., 2002, Grossart *et al*., 2007, Vojvoda *et al*., 2014, Thiele
167 *et al*., 2015, Ivančič *et al*., 2018, Duret *et al*., 2019). Polysaccharides, excreted by
168 bacteria, produce a sticky medium consisting of gel like particles, which provides
169 further structure to the aggregates together with the colloids and organic gels and
170 organic matter (Alldredge *et al*., 1993, Jackson, 1995, Long & Azam, 2001, Passow,
171 2002). Living and lysed cells in the majority of natural environments excrete
172 extracellular polymeric material (Passow, 2002). Dissolved organic matter is removed
173 from the surrounding environment by attached bacteria and converted to particulate
174 matter through extracellular excretion (Alldredge & Silver, 1988).

175 Much like the colonization of surfaces, the colonization of aggregates by
176 bacteria is complex and occurs in several steps. First, bacteria will attach loosely to the
177 aggregate, but the attachment will gradually increase until cells are permanently
178 attached, and growth rates dominate over attachment (Grossart, Kiørboe, Tang & Ploug,
179 2003). Fast swimming bacteria will encounter an aggregate in about <1 day (Kiørboe,
180 Grossart, Ploug, Tang, 2002), but also non-motile bacteria collide with aggregates in
181 lower frequency. Subsequently the total cell numbers on the aggregate increase and the
182 bacterial community becomes established like during the formation of bacteria biofilms
183 on inert surfaces. Although no studies have been conducted to assess the contribution
184 of marine aggregations in fouling of the SWRO membranes, it is known that pre-
185 treatment of the feed water has limitations and not all precursors can be removed. The
186 limited removal of TEP (marine snow) from seawater via pre-treatments increases the
187 biofouling potential (Balzano *et al.*, 2014).

188 **Developmental phases of biofouling**

189 The predominant contributors to biofilm formation on RO membranes are microbial
190 communities and nutrients. The adherence of microbes to the surface of the membrane

and the continual growth of aggregates thus result in the formation of biofilms (Lee *et al*., 2015, Jiang *et al*., 2017, Nagaraj *et al*., 2018).

A new paradigm was proposed by Bar-Zeev *et al.,* (2012) in the role that TEP has alongside the stages of the "classic" formation of biofilm. TEP are often found in marine environment and play a role in the formation and development of marine biofilms (Bar-Zeev *et al*., 2009, Berman, Mizrahi & Dosoretz, 2011, Bar-Zeev, Passow, Castrillón & Elimelech, 2015, Nagaraj *et al*., 2018). Within the desalination process, high levels of potential biofilm forming TEP were found to be reaching the RO membrane (Bar-Zeev *et al*., 2009, Le Lan *et al*., 2015).

The formation of biofilm on the RO membrane involves several key phases:

(I) Conditioning of the membrane surface

(II) Attachment of microorganisms

(III) Formation of biofilm matrix

(IV) Establishment of mature biofilm

(V) Biofilm stability and reduction

Organic and inorganic particles present in the water adhere to the membrane surface forming a nutrient rich 'conditioning film' (Phase I). It is during this phase that TEP precursors absorb to the surface of the membrane subsequently producing a patchy, thin negatively charged conditioning layer (Jain & Bhosle, 2009, Hwang, Liang, Kang, Tong & Liu, 2013, Khan, de O Manes, Aubry, Gutierrez & Croue, 2013, Li *et al*., 2016). Subsequently, increasing the attachment of bacteria through hydrophobic interactions and hydrogen bonding (Hwang *et al*., 2013). 'Protobiofilm' aggregates of free-floating microgels potentially heavily colonised by bacteria, can also adhere to surfaces simultaneously with TEP precursors (Bar-Zeev *et al*., 2012). Once the protobiofilm is attached, it exhibits all the traits common to a mature biofilm and can expedite the

development of biofilms. The initial conditioning of the membrane surface allows microorganisms to adhere due to the nutrient rich environment thereon (Phase II). Van der Waals, hydrophobic and hydrogen bonding allow bacteria are able to overcome electrostatic repulsive forces and reversible attachment of the surface coating (Redman, Walker & Elimelech, 2004, Hori & Matsumoto, 2010). During this phase the application of weak shear forces still has the ability to reverse the attachment to the membrane. The production of EPS by the attached bacteria changes their attachment from reversible to irreversible (Phase III; Dunne, 2002, Hori & Matsumoto, 2010). Simultaneously, micro-gel patches of carbon rich TEP are covering and attaching to the membrane. The EPS layer produced adsorbs the TEP, which then becomes a structural component of the matrix (Barnes *et al*., 2014, Bar-Zeev *et al*., 2015). Thereby, increasing the attachment of bacteria and micro-particles through a more favourable organic content, elasticity and roughness of the membrane surface (Bar-Zeev *et al*., 2012). The surface and the polysaccharides within the EPS interact through hydrophobic interactions, dipole-dipole forces and hydrogen bonding (Dunne, 2002, Hori & Matsumoto, 2010). Organic molecules, colloidal particles, suspended solids and other microorganism cells are captured over time and used to build a thicker matrix layer resulting in a higher permeate flux resistance (Phase IV; Stoodley, Sauer, Davies & Costerton, 2002, Hall-Stoodley, Costerton & Stoodley, 2004). An equilibrium is finally established due to the availability and quantity of the nutrients / food sources also the production of by-products from the bacterial growth process and their subsequent removal and, additionally, the turbulence produced by the cross flow on the surface of the membrane and space limitations (Phase V; Stoodley *et al*., 2002, Hall-Stoodley *et al*., 2004, Gupta *et al*., 2016). The formation of biofouling of SWRO

membranes results in well documented consequences, which can have a detrimental impact on the productivity of the desalination plant (Jiang *et al*., 2017).

Within-system implications of biofouling

The formation of permanent biofilms on RO membranes within a desalination plant is the result of a combination of factors such as the presence of microorganisms within the water, the availability of nutrients and the flow of water through the membrane (Herzberg & Elimelech, 2007, Mo *et al*., 2008, Nagaraj *et al*., 2018). This results in a negative impact on the performance of the system through a decline in the water flux (including permeate), as well as an increase in the amount of seawater rejected, energy requirements, system pressure and also the potential of damage to the RO membrane. The impact of all these leads to a decrease in the quality of freshwater produced (Komlenic, 2010, Matin *et al*., 2011, Katebian & Jiang, 2013, Goh *et al.,* 2018).

Permeate flux decline

Permeate flux decline is directly impacted by the development of biofilm on the RO membranes whether the development is rapid or gradual (Matin *et al*., 2011, Lee *et al*., 2015). Two phases of flux decline have been noted; the initial decline coincides with the early phases of biofilm development especially the attachment and proliferation on the surface of the membrane (Kim *et al*., 2019). The second phase displays a slow decline and has been found to correspond to the final phases in the biofilm development in which equilibrium is developed between biofilm growth, EPS production and biofilm loss (Matin *et al*., 2011, Li *et al*., 2016, Kim *et al*., 2019).

Hydraulic resistance is a direct result of the presence of biofilm and increased osmotic pressure is the result of concentration polarization as a consequence of the complicated biofilm structure (Herzberg & Elimelech, 2007, Chong, Wong & Fane, 2008, Herzberg *et al*., 2009, Kwan, Bar-Zeev & Elimelech, 2015, Ferrando, Ziemba & Herzberg, 2017, Kim *et al*., 2019). Another factor causing a decline in flux is the resistance of the biofilm due to its EPS matrix. Kwan *et al*., (2015) proposed that the resulting shape of the biofilm, due to the pressure exerted upon it, changes the characteristics of the biofilm and thereby causes a greater decline in the flux in RO systems.

Salt rejection

A phenomenon known as 'concentration polarization' is often seen in pressure driven separation processes such as RO desalination (Hoek & Elimelech, 2003, Herzberg & Elimelech, 2007, Herzberg & Elimelech, 2008). The passage of water through the membrane, leads dissolved substrates to accumulate in the feed water resulting in a steady increase in solute concentration in the RO permeate water. The formation of the EPS matrix contributes to the suppression of dissolved solutes mixing at the membrane surface thereby leading to an enhanced concentration polarization (Hoek & Elimelech, 2003, Herzberg & Elimelech, 2007). An increase in the transport of solute material through the membrane is the result of an increase in ionic activity at the surface.

A decline in the rejection of salt in the pressure driven separation system commonly seen in desalination plants is also associated with the growth of biofilm on the membrane (Herzberg & Elimelech, 2007, Radu, Vrouwendelder, van Loosdrecht & Picioreanu, 2012, Ferrando *et al.*, 2017, Siebdrath *et al*., 2019). A study conducted by Herzberg *et al*., (2009) found that the leading cause of an increase in salt passage was

289 the microorganisms within the biofilm and that EPS played only a minor role. However,
290 salt passage was observed by Ferrando *et al.*, (2017) as increasing in parallel with the
291 production of the Pseudomonas aeruginosa EPS component Psl. Thereby, establishing
292 that EPS is a critical factor in not only concentration polarization but also in membrane
293 performance. Within, cellulose acetate membrane systems biodegradation has also been
294 associated with the decrease in the rejection of salt (Beverly, Seal & Hong, 2000,
295 Murphy *et al.*, 2001). Siebdrath *et al.*, (2019) investigated biofouling formation in a
296 long channel membrane test cell pilot plant. While salt rejection decline was evident in
297 all the test cells in series, the decline in the lead cell was the result of biofouling,
298 whereas the decline seen in the tail test cell was a result of high flux decline. However,
299 further insights into the negative impact of biofilms within desalination plants has led
300 to the consideration of biofilm resistance as a concern.
301
302 **Compression and hydraulic resistance of biofilm**
303 The consolidation of biofilms is a process that has recently been defined,
304 implying that under dynamic conditions there is possible restructuring of the biofilm.
305 Compaction is the mechanism contributing to the realignment of the biofilm structure
306 when under high shear force that was proposed by Casey (2007). When compacted, the
307 biofilm increases in density but its porosity decreases, resulting in a decline of biofilm
308 thickness. Biofilm relaxation has the opposite effect resulting in an increase in porosity
309 and, thereby, biofilm thickness. However, it is not yet fully understood how this biofilm
310 behaviour and the resulting impact affect performance loss within desalination plants.
311 The effect of differing fluid velocities on biofilm density was investigated by Ohl, Horn
312 & Hempel, (2004). Increasing the flow velocity sees the biofilms become more
313 compact, and the density of the biofilm decreased with the reduction in flow velocity.

The thickness of the biofilm also determines the time needed to see an impact of the flow velocity; the thicker the biofilm the greater the adaption time.

Currently there are limited studies mechanically exploring the compressibility of biofilms. Körstgens, Flemming, Wingender & Borchard, (2001) investigated the elasticity and yield strength of *P. aeruginosa* biofilms using a film rheometer. Whereas, Paramonova *et al*., (2007) and Paramonova, Krom, van der Mei, Busscher & Sharma, 2009 used low-load compression testing on various bacteria. While these studies provided new reproducible insights into measuring biofilm compressibility, they do not adequately reflect the biofouling parameters. Dreszer *et al*., (2013) investigated the role of biofilm resistance on the performance of MF membranes through different flux and cross flow of drinking water. However, while increased biofilm resistance is seen when the flux increases, the biofilm can be returned to the original resistance when the flux is also reverted back. Dreszer *et al*., (2013) also found that the biofilm had only a small role in the transmembrane resistance due to the intrinsic membrane resistance. They thus suggested that in RO desalination systems, biofilms are likely to enhance biofouling through concentration polarization and feed channel pressure drop rather than pure biofilms. They also proposed that it is the tortuosity of the EPS matrix that leads to the failure of the water to penetrate the matrix. Further, Dreszer *et al*., (2014) undertook an *in-situ* experiment examining the hydraulic resistance in drinking water biofilms and the performance parameters of the MF membrane. Over time with an increase of biofilm thickness, the resistance of the biofilm increased, resulting in a pressure drop within the system. However, the original resistance of the biofilm could not be completely restored with the decrease of flux as evident in their previous study. Dreszer *et al*., (2014) also found that the shape of the biofilm changes as a result of the flux upon it; as the flux increases the biofilm becomes more compact whereas at a lower

permeate flux the biofilm relaxes. The compaction and morphology of the biofilm also have role in the biofouling of the system, as does the thickness. Biofilm modelling conducted by Laspidou, Spyrou, Aravas & Rittmann, (2014) determined that compression of the biofilm 'closed' the channels within resulting in a more ridged biofilm. A study conducted by Valladares Linares *et al*., (2015) determined *in-situ* how flux variations influences biofilm compaction and relaxation. Compaction of the biofilm was seen rapidly after an increase in flux, and although when the original flux was restored the thickness of the biofilm increased, the biofilm was not returned to its initial state. The changes in the biofilm parameters were influenced by the hydraulic conditions leading to a compressed, stiff biofilm, with an increased hydraulic resistance which resulted in a loss of membrane performance. The use of optical coherence tomography did not allow for the determination 'closed' channels as predicted by Laspidou *et al*., (2014), however, the biofilms did increase in stiffness after compression.

Derlon *et al*., (2016) explored the influence that the composition of the biofilm had on hydraulic resistance in gravity-driven membrane ultra-filtration systems. The impact of inorganic materials and cells within the biofilm was determined to be limited due to the minor fraction they represented in the total biofilm volume. However, hydraulic resistance was impacted by biofilms in which EPS was a major component. Derlon *et al*., (2016) also found that the biofilms while compressible they exhibited two responses to a change in transmembrane pressure. The first an immediate response in which, the biofilm relaxes or compresses, upon a sudden change in pressure leads to a change in hydraulic resistance, although the compression was found to be entirely reversible under these conditions. Derlong *et al*., (2016) proposes that these changes are driven the mechanical properties of the biofilm. The second a long-term response in which the

permeability of the biofilm reduces over time increasing hydraulic resistance. Derlon *et al*., (2016) theorises that being under constant pressure causes the biofilm to restructure the internal architecture. Desmond *et al*., (2018) proposed that different compositions of EPS would influence hydraulic resistance in biofilms with a gravity driven membrane ultrafiltration system. Desmond *et al*., (2018) found that the biofilm thickness is relative to the morphology of the EPS, furthermore, the thickness of the biofilm is relative the hydraulic resistance. Desmond *et al*., (2018) determined that biofilms which displayed a homogeneous morphology, containing increased concentrations of polysaccharides and eDNA resulted in a larger volume of functional groups. Resulting in the production of dense structures with reduced permeability, hence, they have a higher hydraulic resistance. The heterogeneous morphology of biofilms was the product of lower concentrations of not only polysaccharides but also eDNA. With greater heterogenicity amongst the functions groups the biofilm produced did not display a density seen in the homogeneous biofilms therefore the hydraulic resistance was not as great. Desmond et al., (2018) proposes that due to the higher concentration of polysaccharides and eDNA this favours the production of a homogeneous structure with high hydraulic resistance due to the close-range electrostatic interactions. With a low concentration of polysaccharides and eDNA thereby limiting the electrostatic interactions with the structure that are required for the formation of a dense biofilm.

Reflection on natural Biofilm formation

The prevalence of biofilms in nature allows microorganisms to survive unforgiving environments while also supporting significant ecological and biogeochemical

functions (Dang & Lovell, 2016, Azeredo *et al.*, 2017). While within the industrial and medical industries biofilms are considered to have a negative impact in particular the ability to resist antimicrobial agents (Lewis, 2001, Azeredo *et al.*, 2017). However, whether being formed on rocks or medical implants the development of biofilms is largely consistent. Biofilms consist of sessile microorganisms contained within a heterogeneous matrix of EPS, which attaches irreversibly to a solid surface. These cells differ from free-living cells of the same species in terms of growth rate and gene expression as they have an altered phenotype (Donlan & Costerton, 2002, Bryers & Ratner, 2004). Physical, chemical and biological processes are involved in guiding the formation of biofilm. Biofilms are considered to be one of the most robust forms of life, and have a strong structure ensuring from the development of EPS layers by the microorganism (Flemming, 2002, Hall-Stoodley *et al*., 2004). A sequence of consecutive stages leads to the development of biofilms such as the initial reversible attachment of microorganism, irreversible attachment of pioneer microorganisms, maturation of biofilm stage I, maturation of biofilm stage II and dispersion (Sauer, Camper, Ehrlich, Costerton, Davies, 2002, Stoodley *et al*., 2002, Gupta *et al*., 2016).

The initial stage of formation of biofilms is heavily dependent upon the transport and attachment of planktonic bacteria by physical, chemical and/or biological factors such as pilli or flagella to a solid-liquid interface (Maric & Vranes, 2007, Armbruster & Parsek, 2018). Biofilm formation is also reliant upon other elements such as surface roughness, hydrodynamics and the characteristics of the surrounding water including pH, nutrient content, ionic strength and multivalent cations biofilms (Vieira, Melo, Pinheiro, 1993, Millsap, Reid, Vandermei, Busscher, 1997, Davies *et al*., 1998, Stoodley, Doods, Boyle, Lappin-Scott, 1999, Sauer & Camper, 2001, Donlan, 2002, Allison, 2003, Parsek & Greenberg, 2005, Chae, Schraft, Hansen, Mackereth, 2006,

414 Herzberg & Elimelech, 2007, Patel, Ebert, Ward, Anderson, 2007, Simões, Pereira,
415 Sillankorva, Azeredo, Vieira, 2007, Oulahal, Brice, Martial, Degrave, 2008, Simões,
416 Simões, Cleto, Pereira, Vieira, 2008). Electrostatic and hydrophobic interactions thus
417 play an important role in attachment as microbes approach the surface (Bos, Van Der
418 Mei, Busscher, 1999, Redman *et al*., 2004, Walker, Redman, Elimelech, 2004, Kang,
419 Subramani, Hoek, Deshusses, Matsumoto, 2004, Schneider *et al*., 2005). Flagella,
420 fimbriae and pili appendages overcome the repulsive forces between the cells and the
421 surface, resulting in a change from reversible to irreversible attachment to the surface
422 during the second stage of biofilm formation (Kumar & Anand, 1998, Garrett, Bhakoo,
423 Zhang, 2008, Tribedi & Sil, 2014, O'Toole & Wong, 2016, Armbruster & Parsek,
424 2018). During the third stage of biofilm development the organisms start to produce
425 biofilm specific genes as a result of communication through autoinducer signals
426 (Davies *et al*., 1998, Vasudevan, 2014, Gupta *et al*, 2016). A strategy for growth and
427 maturation of the biofilm is the production of EPS, which are responsible for the
428 composition, structure and mechanical stability of the matrix that binds microbes and
429 particulate materials together and to surfaces (Stoodley *et al*., 2002, Flemming, Neu,
430 Wozniak, 2007, Simões, Simões, Vieira, 2010, Colvin *et al*., 2011, Franklin, Nivens,
431 Weadge, Howell, 2011).

432 The composition of EPS is more complex than only polysaccharides; proteins,
433 glycoproteins, glycolipids, fibrous proteins, and even extracellular DNA (eDNA) are
434 also found (Whitchurch, Tolker-Nielsen, Ragas, Mattick, 2002, Flemming *et al*., 2007,
435 Herzberg *et al*., 2009, Gloag *et al*., 2013, Erskine *et al*., 2018). The production and
436 composition of EPS is dependent on a number of metabolic processes including growth
437 phase changes, cell lysis, active secretions, macromolecules released from the cell
438 surface and also environmental interactions such as composition, light, pH, and

temperature (Otero & Vincenzini, 2003, Eboigbodin & Biggs, 2008, Quintelas, da Silva, Silva, Figueiredo, Tavares, 2011). The composition of EPS also influences the interactions that microorganisms have with the surrounding environment (Dragoš & Kovács, 2017). Biofilm structural integrity has recently been attributed to fibre-forming proteins which also provide a degree of protection from predation (Blanco *et al*., 2012, Romero & Kolter, 2014, Taglialegna, Lasa, Valle, 2016, Vidokovic *et al*., 2018), while the stability of biofilms is shaped by interactions of the polysaccharide chains within EPS (Higgins & Novak, 1997, Donot, Fontana, Baccou, Schorr-Galindo, 2012). The environment within the biofilm is thus controlled by EPS as it regulates the porosity, density, water content, charge, sorption properties, hydrophobicity and mechanical stability (Flemming *et al*., 2007, Flemming & Wingender, 2010). The physical and chemical properties of EPS at different growth stages are particularly important since EPS determines the ability of the microorganisms or aggregates to trap charged contaminant colloids, adhere to a substrate and resist external forces. The formation of a biofilm allows bacteria a greater defence against desiccation, predation and EPS allows for the capture of nutrients and other minerals that are essential to the survival of the organisms making the biofilm (Flemming & Wingender, 2010, Rasamiravaka, Labtani, Duez, El Jaziri, 2015). During this formation period the biofilm increases in layers and thickness. A major factor shaping the maturation of the biofilm is the physiological cooperation resulting in co-operative microbial communities. (Costerton *et al*., 1995, Strassmann & Queller, 2011, van Gestel *et al*., 2015, Nadell *et al*., 2016). Auto-aggregation and micro-colony formation of the attached cells, as well as the production of EPS ultimately condition the microenvironment within the biofilm (Costerton *et al*., 1995, Herzberg & Elimelech, 2007). The development of the biofilm is dependent on the response of the bacteria (i.e. growth) to the micro-environmental

conditions allowing for the development of a complex mature biofilm (Costerton *et al*., 1995, Davey & O' Toole, 2000, Nadell *et al*., 2016). The physiological cooperation is achieved by channels permeating the biofilm and acting as a circulatory system allowing the microorganisms to exchange water, nutrients, enzymes, signals and to dispose of potentially toxic metabolites (Costerton *et al*., 1995, Davey & O' Toole, 2000, Harrison & Buckling, 2009, Boyle *et al*., 2013, Drescher *et al*., 2014, Nadell et al., 2016, Pollak et al., 2016, Dragoš & Kovács, 2017, Dragoš *et al*., 2018). Acting as a collective also allows the group to influence the surrounding local environment to their own benefit. In the fifth and final stage the biofilm disperses through shedding releasing the sessile organisms (Hall-Stoodley *et al*., 2004). Within the biofilm, the microorganism community disrupts the matrix stabilizing polysaccharides through the production of various saccharolytic enzymes releasing the residing bacteria. To enable translocation, the expression of flagella proteins is upregulated prior to release allowing for the spreading of the biofilm through the colonisation of new surfaces (Gupta *et al*., 2016).

While the process of basic biofilm formation provides insights into the formation of biofilms on RO membranes, however, other factors influence biofouling:

- The availability of biofilm precursors in the seawater
- The formation of aggregates within the desalination system.
- The inflow of nutrients to the biofilm.
- The pressure of the system driving aggregates, bacteria and nutrients into the RO membrane.

Conclusions and future area of study

Biofouling is a widely recognised issue in membrane-based system including desalination plants. The RO membrane within saltwater desalination plants are one of the components that is significantly affected by biofouling. The inflow of live biofilm forming bacteria, organics and nutrients onto the RO membrane allows for growth and proliferation of biofilms. The fouling of membranes has detrimental consequences including increased hydraulic resistance, enhanced concentration polarization, decreased salt rejection and decreased membrane permeability. As the membrane becomes further fouled, the performance deteriorates impacting not only the life span of the membrane but also the production of water and the systems energy requirements consequently increasing fiscal requirements. Hence, the development of methods to reduce the impact that biofouling has on RO membranes is of the utmost importance.

Biofilms are found to be ubiquitous in most environments. The basic stages in the formation of biofilms are mostly well understood, however, there is still some debate about the determining factors such as genetic or environmental influences that contribute to biofilm formation. Being embedding in a matrix of EPS provides structure and protection to the biofilm, the viscoelastic nature of EPS allows them to adjust to different environmental conditions. The marine environment and thereby the feed water has a substantial role in biofouling. As the in-flow contains all the components that make biofouling possible and deposits them on the RO membrane. It is the DOM and micro and macro aggregates that consist of or contain TEP that are the initiators of biofilm the membranes. Thereby, changing the process of biofilm formation. The negative impact seen as a result of biofilms on the RO membranes reducing the ability of seawater to pass through. Both microorganism and EPS have been attributed to a decline in the permeate flux and but only microorganisms influence the decrease of salt rejection with EPS only participating slightly. Biofilm compaction is seen under

513 increased permeate flux and influences the structure of the biofilm. Upon increased
514 hydraulic conditions biofilms become compressed and stiff as a result of this over time
515 hydraulic resistance is increased leading to reduced membrane performance.

516 With many different aspects involved in biofouling improving control strategies
517 is essential to maintain a cost -effective desalination plant; TEP is recognised as a key
518 component in biofouling and should be focused on when researching improved ways
519 to control biofouling. Recommendations for future research are as follow:

520 • Interactions between the membrane, TEP and microorganisms offer insights to
521 improve on novel membrane surface modification. Further information is required
522 before control strategies for biofilm control are truly effective.
523 • Improved knowledge of the physicochemical nature of TEP could provide insights
524 not only for reducing the biofilm via novel cleaning treatments but also in devising
525 pre-treatments that could remove or reduce the amount in the feed water.
526 • Better understanding of biofilm formation, morphology, compression and relaxation
527 especially during crossflow velocity and permeate flux would greatly enhance
528 treatment capabilities.

529 Investigations into the viscoelasticity of EPS, since it has been determined that it is
530 responsible an increase in hydraulic resistance and membrane performance, is critical.

531

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