From biofilm ecology to reactors: a focused review

Joshua P. Boltz, Barth F. Smets, Bruce E. Rittmann,
Mark C. M. van Loosdrecht, Eberhard Morgenroth and Glen T. Daigger

ABSTRACT

Biofilms are complex biostructures that appear on all surfaces that are regularly in contact with water. They are structurally complex, dynamic systems with attributes of primordial multicellular organisms and multifaceted ecosystems. The presence of biofilms may have a negative impact on the performance of various systems, but they can also be used beneficially for the treatment of water (defined herein as potable water, municipal and industrial wastewater, fresh/brackish/salt water bodies, groundwater) as well as in water stream-based biological resource recovery systems. This review addresses the following three topics: (1) biofilm ecology, (2) biofilm reactor technology and design, and (3) biofilm modeling. In so doing, it addresses the processes occurring in the biofilm, and how these affect and are affected by the broader biofilm system. The symphonic application of a suite of biological methods has led to significant advances in the understanding of biofilm ecology. New metabolic pathways, such as anaerobic ammonium oxidation (anammox) or complete ammonium oxidation (comammox) were first observed in biofilm reactors. The functions, properties, and constituents of the biofilm extracellular polymeric substance matrix are somewhat known, but their exact composition and role in the microbial conversion kinetics and biochemical transformations are still to be resolved. Biofilm grown microorganisms may contribute to increased metabolism of micro-pollutants. Several types of biofilm reactors have been used for water treatment, with current focus on moving bed biofilm reactors, integrated fixed-film activated sludge, membrane-supported biofilm reactors, and granular sludge processes. The control and/or beneficial use of biofilms in membrane processes is advancing. Biofilm models have become essential tools for fundamental biofilm research and biofilm reactor engineering and design. At the same time, the divergence between biofilm modeling and biofilm reactor modeling approaches is recognized.

Key words | aerobic granular sludge, biofilm, ecology, integrated fixed-film activated sludge, membrane-supported biofilm reactors, moving bed biofilm reactor

Joshua P. Boltz
Volkert, Inc.,
3809 Moffett Road,
Mobile,
AL 36618,
USA

Barth F. Smets
Department of Environmental Engineering,
Technical University of Denmark,
Miljøvej 113,
2800 Kgs. Lyngby,
Denmark

Bruce E. Rittmann
Swette Center for Environmental Biotechnology,
Biodesign Institute at Arizona State University,
P.O. Box 875701,
Tempe,
AZ 85287-5701,
USA

Mark C. M. van Loosdrecht
Department of Biotechnology, Faculty of Applied Sciences,
Delft University of Technology,
Van der Maasweg 9,
2629 HZ Delft,
The Netherlands

Eberhard Morgenroth
ETH Zürich, Institute of Environmental Engineering,
8093 Zürich,
Switzerland
and
Eawag, Swiss Federal Institute of Aquatic Science and Technology,
8600 Dübendorf,
Switzerland

Glen T. Daigger (corresponding author)
University of Michigan,
1351 Beal Ave.,
Ann Arbor,
MI 48109,
USA
E-mail: *gdaigger@umich.edu*

INTRODUCTION

Biofilms are complex biostructures which appear on all surfaces that are regularly in contact with water. A biofilm consists of prokaryotic cells and other microorganisms such as yeasts, fungi, and protozoa that secrete a mucilaginous protective coating in which they are encased (i.e., extracellular polymeric substances or EPS). Biofilms can form on solid or liquid surfaces as well as on soft tissue in living organisms. Biofilms are typically highly resilient constructs that resist conventional methods of disinfection. Biofilm formation is an ancient and integral component of the prokaryotic life cycle, and it is a key factor for survival in diverse environments. Biofilms are structurally complex, dynamic systems with attributes of both primordial multicellular organisms and multifaceted ecosystems. The formation of biofilms represents a protected mode of bacterial growth that allows cells to survive in hostile

doi: 10.2166/wst.2017.061

environments and disperse to colonize new niches (Hall-Stoodley *et al.* 2004).

The presence of biofilms may have a negative impact on the performance of various systems. For example, biofouling of ship hulls and membrane surfaces reduces performance and efficiency, resulting in marked financial costs. Pathogenic biofilms have also proven detrimental to human health. Biofilm infections, such as pneumonia in cystic fibrosis patients, chronic wounds, chronic otitis media, and implant- and catheter-associated infections, affect millions of people in the developed world each year, and many deaths occur as a consequence (Bjarnsholt 2013). Foodborne diseases – often caused by biofilm-forming pathogens – are a public health concern throughout the world (Srey *et al.* 2013). The development of multispecies biofilms on teeth (i.e., dental plaque), and their associated bacterial pathogenesis, can lead to gum disease and tooth decay (Kolenbrander *et al.* 2010). Biofilms may also be undesirable in the open water environment. For example, algal mat formation on water bodies is a component of the eutrophication process. Finally, biofilms that develop on the interior walls of pipes that comprise a potable water distribution system can lead to additional chlorine demand, coliform growth, pipe corrosion, poor water taste, and foul odor (Hallam *et al.* 2001).

On the other hand, biofilms, can be controlled and used beneficially for the treatment of water (defined herein as potable water, municipal and industrial wastewater, fresh/brackish/salt water bodies, groundwater) as well as in water stream-based biological resource recovery systems. The investigation of biofilms in the water environment will be classified for the purpose of this review into three major categories: (1) biofilm ecology, (2) biofilm reactor technology and design, and (3) biofilm modeling. Biofilm ecology is defined here as the study of components and processes that take place in the biofilm. Biofilm reactor technology and design encapsulates the development, design, operation, and optimization of bioreactors that target controlled biofilm utilization. Biofilm modeling is the development and application of various computational approaches to simulate, predict, or synthesize the processes occurring in biofilms and biofilm reactors.

The term biofilm refers to the microbes and associated deposits on a surface embedded in the matrix of EPS. The broader term, biofilm system, includes other components affecting the biofilm, and usually consists of, at least, the substratum (on which the biofilm forms) and the bulk phase (which flows over the biofilm). This paper reviews key research and practical events related to these areas of biofilm study, focusing on research and practice-related trends in biofilm-related biology, biofilm reactors, and models of particular relevance to challenges and opportunities regarding biofilms and biofilm systems.

BIOFILM BIOLOGY: METHODS TO ECOLOGY

The biology of biofilms includes a diverse array of topics. The current focus of biofilm biology is dedicated to applying state-of-the-art approaches to evaluate biofilm ecology in relation to structure and function, including the identification of factors that drive biofilm formation and dispersal.

The symphonic application of biological methods is essential to understand microbial films biology. The currently and often used combined application of quantitative polymerase chain reaction (qPCR), fluorescent *in situ* hybridization (FISH), advanced 2-D microscopy, and micro-scale chemical sensors has allowed biofilm researchers to create a better vision of biofilm make-up – including both the cellular matter and their excretions – than ever before. This insight has proven valuable to advancing the understanding of biofilm structure and function. qPCR has been used to further our understanding of biofilm structure and function, and the roles that biofilms play in a bioreactor (Kim *et al.* 2011). Applying qPCR combined with microdissection has allowed one to quantify the stratification of functional guilds in biofilms (Terada *et al.* 2010). FISH is a technique that is based on hybridizing a fluorescently labelled DNA probe to (typically for bacterial investigations) complementary sequences present in the bacterium's 16S rRNA. Phylogenetically distinct groups of bacteria can be simultaneously visualized by proper choice of DNA probes. When properly applied to biofilms – and in combination with the right microscopic method and detection method (often multi-channel confocal laser scanning microscopy (CLSM)) the technique allows one to identify the spatial organization and relative location of different bacterial groups (Okabe *et al.* 1999; Vlaeminck *et al.* 2010). CLSM, transmission electron microscopy, and soft X-ray scanning transmission X-ray microscopy have been used to map the distribution of macromolecular sub-components (e.g., polysaccharides, proteins, lipids, and nucleic acids) of biofilm cells and their associated EPS matrix (Lawrence *et al.* 2003). More recently, optical coherence tomography has been applied to visualize the mesoscale structure of biofilms (Wagner *et al.* 2010), and confocal Raman spectroscopy has provided a tool for studying the chemical heterogeneities of biofilms *in situ* (Sandt *et al.* 2007).

Microbial ecology is an essential component of biofilm studies because of the desire to control biofilm development, biochemical transformation processes, and dispersion. Davies *et al.* (1998) suggested that a cell-to-cell signal is involved in the development of *Pseudomonas aeruginosa* biofilms. These findings implied involvement of an intercellular signal molecule in the growth of *P. aeruginosa* biofilms, which suggests possible targets to control biofilm growth, for example, on catheters, in cystic fibrosis, and in other environments where problematic *P. aeruginosa* biofilms persist. Shrout *et al.* (2006) documented the impact of quorum sensing and swarming motility on *P. aeruginosa* biofilm formation as being nutritionally conditional. Nitric oxide (NO) is an important gaseous messenger molecule in a biological system that is produced by one cell, penetrates through membranes, and regulates the function of another cell (Zetterström 2009). This discovery presented an entirely new principle for signaling in biological systems. Various NO donors of clinical and industrial significance have been demonstrated viable, in a laboratory system, for dispersal in single- and multispecies biofilms (Barraud *et al.* 2009). Applications in natural formed biofilms have, however, not yet been reported.

Research on biofilm reactors has been the source of an interesting new metabolic pathway. The anaerobic ammonium oxidation (anammox) process was discovered in a pilot-scale denitrifying fluidized bed biofilm reactor. From this system, a highly enriched microbial community was obtained, dominated by a single deep-branching planctomycete, *Candidatus* Brocadia anammoxidans (Jetten *et al.* 2001). Since that time, the utilization of anammox microorganisms in biofilm reactors has proven popular, cost effective, and efficient.

The continued development of knowledge about phototrophic biofilms has elucidated their utility for nutrient removal from wastewater, heavy metal accumulation and water detoxification, oil degradation, agriculture, aquaculture, and sulfide removal from contaminated waste streams (Roeselers *et al.* 2008).

Microorganisms in biofilms live in a self-produced gelatinous matrix of EPS, consisting primarily of polysaccharides, proteins, nucleic acids and lipids. EPS provide biofilms with mechanical stability, mediates bacterial adhesion to surfaces, and serves as the three-dimensional polymer network that interconnects and transiently immobilizes bacterial cells inside a biofilm. EPS are also capable of entrapping, or bioflocculating, biodegradable and non-biodegradable particulates in the polymeric matrix (Boltz & LaMotta 2007). Conceptually, some basic functions, properties and constituents of the EPS matrix are known, but the kinetics of EPS production and consumption, their contribution to the conversion of materials entrapped within them, and their contribution to metabolic kinetics and biochemical transformation rates owing to microbial growth in a biofilm are poorly defined. Thus, biofilm models explicitly describing EPS (e.g., Alpkvist *et al.* 2006; Celler *et al.* 2014) are scarce and lack the measurement (i.e., quantification) of fundamental mechanical properties. Hence, unlocking this not yet well-defined aspect of biofilms remains a challenge to researchers (Flemming & Wingender 2010). EPS play an important role in biofilms, including the agglomeration of selenium (Gonzalez-Gil *et al.* 2016). EPS extraction methods are still not well validated (Pellicer-Nàcher *et al.* 2013a, 2013b); several types of EPS matrix polymers are not solubilized in standard extraction methods (Lin *et al.* 2013), whereas the methods to measure polysaccharides and proteins easily give biased results (Le & Stuckey 2016).

The uptake and biochemical transformation of micro-constituents (including pharmaceuticals) that can occur during wastewater treatment (Jelic *et al.* 2011) is still a significant challenge to biofilm researchers and treatment system designers. Kim *et al.* (2009) compared the removal efficiencies of micro-constituents classified as trace organic chemicals (including endocrine disrupting compounds (EDCs) and estrogenic activity). Results suggest that the system with a biofilm compartment out-performed the suspended growth control process. Thus, bioreactors having a biofilm compartment, such as integrated fixed-film activated sludge (IFAS) systems, may be beneficial for enhancing the removal of estrogens and at least some trace organics. These researchers found further evidence for removal by heterotrophic biodegradation, rather than by sorption or removal by nitrifiers. This is significant, given the apparent correlation of ammonia-nitrogen oxidation with the metabolism of specific EDCs, while the biochemical transformation of other EDC types fails to correlate with nitrification. Torresi *et al.* (2016) suggest that biofilm thickness influences the biodiversity of nitrifying biofilms grown in moving bed biofilm reactors (MBBRs), and that this parameter influences a biofilm's capacity for micro-pollutant removal. The biochemistry and microbiology of micro-pollutant transformation – in context of biofilms – is under active investigation, and the identification of the responsible organisms, the role of different functional guilds, the contribution of co- vs primary metabolisms, and the significance of biofilm redox conditions are all under examination.

REACTORS

Biofilms can be controlled and harnessed to provide the basis for their utilization for water treatment via biofilm

reactors. The presence of biofilms may also be undesirable in a biological water treatment system, however, and can cause operational difficulties that increase the expense of treatment.

Biofilm reactors: the beneficial use of biofilms

Biofilm reactors represent the primary means to harness the usefulness of biofilms for the treatment of water(s). Biofilms in these reactors serve as a principal mechanism for the biological transformation of nutrients that are regarded as environmental pollutants (e.g., biodegradable organic matter, nitrogen, and phosphorus). Several types of biofilm reactors have been utilized for water treatment, but currently much focus is on MBBRs and IFAS processes, membrane-supported biofilm reactors (MBfRs), and granular processes.

MBBRs and IFAS processes are mature technologies that continue to evolve. State-of-the-art MBBRs and IFAS processes use submerged free-moving biofilm carriers and can be used for carbon oxidation, nitrification, denitrification, and deammonification (Rusten *et al.* 2006; McQuarrie & Boltz 2011; Odegaard *et al.* 2014). Recent research has offered expanded insight into the role of these biofilm carrier types on mass transfer, and the impact of hydrodynamics on related biochemical transformation processes (Herrling *et al.* 2014; Melcer & Schuler 2014). Globally, there are more than 1,200 full-scale, operating MBBRs having a capacity of 200 population equivalent (p.e.) or greater. It is estimated that approximately 25% of these units are IFAS. MBBRs having a capacity less than 200 p.e. are numbered more than 7,000, globally. More than 100 MBBRs exist for nitrification in aquaculture. It is estimated that there is an equal distribution of MBBRs amongst industrial and municipal wastewater treatment facilities designed to treat waste streams for p.e. greater than 200. The geographic distribution of these installations is estimated as:

- facilities greater than 200 p.e. – 40% in Europe, 30% in North America, 20% in continental Asia and the South Pacific (not including India), and 10% in Africa;
- facilities less than 200 p.e. (including onsite facilities) – 80% in Europe, 10% in North America, and 10% in continental Asia and the South Pacific (not including India).

An MBBR-based process at the Lillehammer wastewater treatment plant (WWTP), Lillehammer, Norway, for the treatment of municipal wastewater has been described by Rusten *et al.* (1995) and an example IFAS installation has been documented at the Fields Point Wastewater Treatment Facility, Rhode Island, USA. The MBBR is an effective platform for simultaneous partial nitritation and deammonification. The AnitaMOX™ process is a commercially available system that has MBBR/IFAS appurtenances and exploits the partial nitritation/anammox process (PN/A) (Veuillet *et al.* 2014). A full-scale AnitaMOX system exists at the Sjölunda WWTP, Malmö, Sweden (Christensson *et al.* 2013).

Granular biomass development and utilization in a sequencing batch reactor (SBR) has proven an effective and highly promising environmental biotechnology for the treatment of contaminated water streams. Aerobic granules can be formed and maintained in SBRs (de Kreuk *et al.* 2007). The potential for stable aerobic granule formation was reported by Beun *et al.* (1999). Currently, more than 25 WWTPs are operating or under construction on four continents, including Europe (five in the Netherlands), South America, Africa and Australia, that will utilize aerobic granular biomass processes. All of these WWTPs are designed for biological nutrient removal from municipal wastewaters. The largest capacity constructed to date has a capacity 517,000 p.e., with an average daily flow of 55,000 m^3/day, in Rio de Janeiro, Brazil. A commercially available aerobic granular sludge system that has been used for successful biological nutrient removal from screened/degritted wastewater or primary effluent is named NEREDA™. A full-scale NEREDA process at Garmerwolde WWTP, The Netherlands, has been described in the literature (Pronk *et al.* 2015). The NEREDA process maintains a constant liquid/biomass volume. The filling, settling, and decanting steps occur simultaneously during approximately 25–33% of the operational period. The remainder of operation is reserved for aeration (i.e., reaction period). Approximately 10–15 minutes is required to achieve reactor quiescence before a next cycle can start with influent feeding from the bottom. These typical operational parameters, along with appropriate influent wastewater characteristics, result in effluents having TN <5 g/m^3 and TP <1 g/m^3. These simple bioreactors are, essentially, an empty tank with fine-bubble aeration and an influent wastewater distribution system along the tank bottom. The treated effluent flows over an effluent weir situated along the top of the tank. The bioreactor has no mixers, but does have an effluent discharge via overflow weirs and a waste sludge collection system (which is situated near the top of the settling sludge bed to promote wasting of more slowly settling sludge). Another approach to benefit from granular biomass is to use a cyclone or screens for the selective retention of granular biomass. Granules have also been used for PN/A

systems of high ammonia-nitrogen concentration waste streams from digested sludge dewatering and anaerobically treated wastewater in processes such as ANAMMOX™ (van der Star *et al.* 2007) and DEMON™ (Wett 2007).

Another biofilm reactor type that exhibits great potential is the MBfR. The potential diverse range of applications for this process is a formidable strength. Gas-delivery to the liquid phase in these systems happens by means of a membrane (tubular, hollow-fiber, or flat) on which the biofilm directly grows. Electron donor and electron acceptor are subject to counter-diffusion through the biofilm from the bulk of the liquid and from the membrane lumen. Two systems have been promoted: (1) the hydrogen-based MBfR (Rittmann 2006), which delivers hydrogen as electron donor to a biofilm, and (2) the oxygen/air-based MBfR (Syron & Casey 2008), which delivers oxygen as electron acceptor to the biofilm. The latter is also known as the membrane aerated biofilm reactor (Martin & Nerenberg 2012). Hydrogen-based MBfRs have been demonstrated viable for the biochemical transformation of nitrate, nitrite, perchlorate, bromate, selenate/selenite, arsenate, and chromate to name only some. As the MBfR allows for a higher control of electron donor/acceptor delivery, biofilms with defined or strong redox stratification can be developed for a simultaneous oxic/anoxic process such as nitritation/anammox. Commercially available MBfRs exist. The membrane aerated biofilm reactor may be procured in North America as the ZeeLung™ process (Côte *et al.* 2015), and in Ireland as the OxyMem™ process. These processes are well suited for combined carbon oxidation and nitrification, nitrification, denitrification, partial nitritation and deammonification. A single unit demonstrating the ZeeLung™ system exists, treating approximately 2,300 p.e. for tertiary nitrification at the O'Brien Water Reclamation Plant, Chicago, Illinois, USA. At least nine full-scale OxyMem processes exist, collectively treating a ranging of flows and meeting a diverse array of treatment objectives throughout Japan, Sweden, Spain, United Kingdom, Ireland, and Brazil.

The continuous enhancement to and implementation of new water quality regulations, and the discovery of new processes, have made mature biofilm reactor types relevant to current trends and challenges that face this community. For example, the US Environmental Protection Agency has enacted a primary drinking water standard that requires selenium concentrations to be less than 0.05 mg/L. This regulation has impacted the agriculture, mining, and power (coal and oil) industries, to name a few. The use of expensive reagents and the production of hazardous residues make the use of physicochemical treatment impractical. As a result, the biological transformation of selenate and selenite to elemental selenium is preferred. Biofilm reactors capable of operating under anaerobic conditions are required; hence particulate biofilm reactors, as described by Nicolella *et al.* (2000), are of renewed interest. Similarly, processes such as SANI (Wang *et al.* 2009) and DEAMOX (Kalyuzhnyi *et al.* 2006) have made use of biofilm reactors such as deep-bed filters and upflow anaerobic sludge blankets.

Finally, biofilms have recently been thoroughly investigated for their capacity to biologically generate electricity, the so-called microbial fuel cell (MFC). Liu *et al.* (2004) demonstrated that MFCs can produce electricity while biologically converting complex compounds present in municipal wastewater. There are several different means for constructing an MFC. Logan *et al.* (2006) presented means for constructing MFCs, compared devices on an equivalent basis, and reviewed an array of related scientific principles, ranging from environmental engineering to microbiology and electrochemistry. The creation of an MFC that can yield sufficient electrical output for economically viable production and utilization eludes researchers, and remains a challenge for biofilm scientists and engineers. MFCs, when operated in the electrolysis mode as microbial electrolysis cells, can produce useful chemical products such as hydrogen and ethanol (Zhou *et al.* 2013).

Unwanted biofilms: toward control

The deleterious role of biofilms on membranes is also an area of concern to process designers and biofilm researchers. Membrane biofouling is a costly operational concern, for example as a feed-spacer problem in spiral-wound membranes (Vrouwenvelder *et al.* 2009). The role that quorum sensing plays in dispersing biofilms has led biofilm researchers to seek membrane biofouling control measures via quorum sensing (Yeon *et al.* 2009). Alternatively, Vrouwenvelder *et al.* (2011) presented a scenario for controlling spiral-wound membrane biofouling by reducing flow pace, modified feed-spacer design, and an advanced cleaning strategy. Another approach to dealing with undesired biofilms that grow on membranes is to tolerate their existence, and focus on increasing hydraulic conductivity of the growing biofilms rather than trying to prevent their formation; ultimately one may benefit from the biological activity in a biofilm to improve permeate quality (Chomiak *et al.* 2015).

BIOFILM MODELING

Biofilm models are essential both to the study and development of fundamental biofilm research and the development

and implementation of biofilm reactors (Morgenroth *et al.* 2000). A consensus description and comparison of biofilm models was presented by Wanner *et al.* (2006). This effort led to the widespread development and application of one-dimensional biofilm models as an engineering tool (Boltz *et al.* 2010). Nevertheless, multi-dimensional models (e.g., Picioreanu *et al.* 2004) have enhanced virtually every form of biofilm research and system development. A clear dichotomy has existed between the use of biofilm models as a research resource and the more recent use as an engineering tool. Bioreactor hydrodynamics has a substantial influence on the degree of uncertainty that is affiliated with the use of mechanistic biofilm models to describe a biofilm reactor (Boltz & Daigger 2010). Therefore, accounting for the importance of bulk-liquid hydrodynamics and system idiosyncrasies (e.g., biofilm carrier type and transport) via simulation has become an integral consideration for biofilm and biofilm reactor modelers (Kagawa *et al.* 2015; Boltz *et al.* (submitted)). Biofilm models have become an increasingly important tool for biofilm researchers and biofilm reactor designers who are interested in the most relevant topics in environmental biotechnology, including greenhouse gas emissions (Van Hulle *et al.* 2012; Sabba *et al.* 2017), phototrophic biofilms (Wolf *et al.* 2007), biofouling in membrane separation systems (Radu *et al.* 2015) and MFCs (Picioreanu *et al.* 2007).

CLOSING COMMENTS

Fundamental principles describing biofilms exist as a result of focused research, practical application, and modeling. The use of reactors for the treatment of municipal and industrial wastewaters is a common beneficial use of biofilms. Applied research exists that provides a basis for the mechanistic understanding of biofilm systems. The empirical information derived from such applied research has been used to develop design criteria for biofilm reactors and remains the basis for the design of many biofilm reactor types despite the emergence of mathematical models as reliable tools for research and practice. There is a gap between our current understanding of biofilm fundamentals and reactor-scale empirical information, represented by the dichotomy in the literature between our knowledge about and use at the micro-scale (biofilm) and macro-scale (reactor). Lewandowski & Boltz (2011) highlighted this division by describing state-of-the-art basic research and practice oriented beneficial use of biofilm systems for the sanitation of water.

ACKNOWLEDGEMENTS

The contributions of the following colleagues to both our knowledge of biofilms and biofilm reactors and the contents of this review are gratefully acknowledge: Jeff Peeters, General Electric Water Division; Eoin Syron, OxyMem; Kim Sørensen, WABAG; Robert Nerenberg, University of Notre Dame, Hallvard Odegaard, NTU. This manuscript is based on a contribution by the authors in the IWA report *Global Trends & Challenges in Water Science, Research and Management* and is published with IWA's permission.

REFERENCES

Alpkvist, E., Picioreanu, C., van Loosdrecht, M. C. M. & Heyden, A. 2006 Three-dimensional biofilm model with individual cells and continuum EPS matrix. *Biotechnol. Bioeng.* **94** (5), 961–979.

Barraud, N., Storey, M. V., Moore, Z. P., Webb, J. S., Rice, S. A. & Kjelleberg, S. 2009 Nitric oxide-mediated dispersal in single- and multi-species biofilms of clinically and industrially relevant microorganisms. *Microb. Biotechnol.* **2** (3), 370–378.

Beun, J. J., Hendriks, A., van Loosdrecht, M. C. M., Morgenroth, E., Wilderer, P. A. & Heijnen, J. J. 1999 Aerobic granulation in a sequencing batch reactor. *Water Res.* **33** (10), 2283–2290.

Bjarnsholt, T. 2013 The role of bacterial biofilms in chronic infections. *APMIS Suppl.* **136**, 1–51.

Boltz, J. P. & Daigger, G. T. 2010 Uncertainty in bulk-liquid hydrodynamics and biofilm dynamics creates uncertainties in biofilm reactor design. *Water Sci. Technol.* **61** (2), 307.

Boltz, J. P. & LaMotta, E. J. 2007 Kinetics of particulate organic matter removal as a response to bioflocculation in aerobic biofilm reactors. *Water Environ. Res.* **79** (7), 725–735.

Boltz, J. P., Morgenroth, E. & Sen, D. 2010 Mathematical modelling of biofilms and biofilm reactors for engineering design. *Water Sci. Technol.* **62** (8), 1821–1836.

Boltz, J. P., Johnson, B. R., Takács, I., Daigger, G. T., Morgenroth, E., Brockmann, D., Kovács, R., Calhoun, J. M., Choubert, J.-M. & Derlon, N. (submitted) Biofilm carrier migration model describes reactor performance. *Water Sci. Technol. Modelling Seminar (WWTmod 2016), Annecy, France.*

Celler, K., Hodl, I., Simone, A., Battin, T. J. & Picioreanu, C. 2014 A mass-spring model unveils the morphogenesis of phototrophic biofilms. *Sci. Rep.* **4**, 3649.

Chomiak, A., Traber, J., Morgenroth, E. & Derlon, N. 2015 Biofilm increases permeate quality by organic carbon degradation in low pressure ultrafiltration. *Water Res.* **85**, 512–520.

Christensson, M., Ekström, S., Andersson Chan, A., Le Vaillant, E. & Lemaire, R. 2013 Experience from start-ups of the first ANITAMox plants. *Water Sci. Technol.* **67** (12), 2677–2684.

Côte, P., Peeters, J., Adams, N., Hong, Y., Long, Z. & Ireland, J. 2015 A new membrane-aerated biofilm reactor for low-energy wastewater treatment: pilot results. In: *Proceedings of the Water Environment Federation Technical Exhibition and Conference (WEFTEC) 2015.*

Davies, D. G., Parsek, M. R., Pearson, J. P., Iglewski, B. H., Costerton, J. W. & Greenberg, E. P. 1998 The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* **280** (5361), 295–298.

De Kreuk, M. K., Kishida, N. & van Loosdrecht, M. C. M. 2007 Aerobic granular sludge – state of the art. *Water Sci. Technol.* **55** (8–9), 75–81.

Flemming, H.-C. & Wingender, J. 2010 The biofilm matrix. *Nat. Rev. Microbiol.* **8**, 623–633.

Gonzalez-Gil, G., Lens, P. N. & Saikaly, P. E. 2016 Selenite reduction by anaerobic microbial aggregates: microbial community structure, and proteins associated to the produced selenium spheres. *Front. Microbiol.* **7** (571), 1–14.

Hallam, N. B., West, J. R., Forster, C. F. & Simms, J. 2001 The potential for biofilm growth in water distribution systems. *Water Res.* **35** (17), 4063–4071.

Hall-Stoodley, L., Costerton, J. W. & Stoodley, P. 2004 Bacterial biofilms: from the natural environment to infectious diseases. *Nat. Rev. Microbiol.* **2**, 95–108.

Herrling, M. P., Guthausen, C., Wagner, M., Lackner, S. & Horn, H. 2014 Determining the flow regime in a biofilm carrier by means of magnetic resonance imaging. *Biotechnol. Bioeng.* **112** (5), 1023–2032.

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sanchez, F., Petrovic, M. & Barcelo, D. 2011 Occurrence, partition, and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. *Water Res.* **45** (3), 1165–1176.

Jetten, M. S. M., Wagner, M., Fuerst, J., van Loosdrecht, M. C. M., Kuenen, G. & Strous, M. 2001 Microbiology and application of the anaerobic ammonium oxidation (anammox) process. *Curr. Opin. Biotechnol.* **12** (3), 283–288.

Kagawa, Y., Tahata, J., Kishida, N., Matsumoto, S., Picioreanu, C., van Loosdrecht, M. C. M. & Tsuneda, S. 2015 Modeling the nutrient removal process in aerobic granular sludge system by coupling the reactor- and granule-scale models. *Biotechnol. Bioeng.* **112** (1), 53–64.

Kalyuzhnyi, S., Gladchenko, M., Mulder, A. & Versprille, B. 2006 DEAMOX – new biological nitrogen removal process based on anaerobic ammonia oxidation coupled to sulphide-driven conversion of nitrate into nitrite. *Water Res.* **40** (19), 3637–3645.

Kim, H.-S., Pei, R., Cho, D., Gellner, J., Boltz, J. P., Gunsch, C., Freudenberg, R. & Schuler, A. J. 2009 Comparison of trace organics and estrogenic activity removal in integrated fixed-film activated sludge and conventional wastewater treatment. In: *Proceedings of the Water Environment Federation, Microconstituents and Industrial Water Quality Conference*. Vol. 12, pp. 319–330.

Kim, H.-S., Schuler, A. J., Gunsch, C. K., Pei, R., Gellner, J. W., Boltz, J. P., Freudenberg, R. G. & Dodson, R. 2011 Comparison of conventional and integrated fixed-film activated sludge systems: attached- and suspended-growth functions and polymerase chain reaction measurements. *Water Environ. Res.* **83** (7), 627–635.

Kolenbrander, P. E., Palmer Jr, R. J., Periasamy, S. & Jakubovics, N. S. 2010 Oral multispecies biofilm development and the key role of cell-cell distance. *Nat. Rev. Microbiol.* **8**, 471–480.

Lawrence, J. R., Swerhone, G. D. W., Leppard, G. G., Araki, T., Zhang, X., West, M. M. & Hitchcock, A. P. 2003 Scanning transmission X-ray, laser scanning, and transmission electron microscopy mapping of the exopolymeric matrix of microbial biofilms. *Appl. Environ. Microbiol.* **69** (9), 5543–5554.

Le, C. & Stuckey, D. C. 2016 Colorimetric measurement of carbohydrates in biological wastewater treatment systems: a critical evaluation. *Water Res.* **1** (94), 280–287.

Lewandowski, Z. & Boltz, J. P. 2011 Biofilms in water and wastewater treatment. In: *Treatise on Water Science*, Vol. 4 (P. Wilderer, ed.). Academic Press, Oxford, pp. 529–570.

Lin, Y. M., van Loosdrecht, M. C. M. & Sharma, P. K. 2013 The chemical and mechanical differences between alginate-like exopolysaccharides isolated from aerobic flocculent sludge and aerobic granular sludge. *Water Res.* **47** (2013), 57–65.

Liu, H., Ramnarayanan, R. & Logan, B. E. 2004 Production of electricity during wastewater treatment using a single chamber microbial fuel cell. *Environ. Sci. Technol.* **38** (7), 2281–2285.

Logan, B. E., Hamelers, B., Rozendal, R., Schroder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W. & Rabaey, K. 2006 Microbial fuels cells: methodology and technology. *Environ. Sci. Technol.* **40** (17), 5181–5192.

Martin, K. J. & Nerenberg, R. 2012 The membrane biofilm reactor (MBfR) for water and wastewater treatment: principles, applications, and recent developments. *Bioresour. Technol.* **122**, 83–94.

McQuarrie, J. P. & Boltz, J. P. 2011 Moving bed biofilm reactor technology: process applications, design, and performance. *Water Environ. Res.* **83** (6), 560–575.

Melcer, H. & Schuler, A. J. 2014 *Mass Transfer Characteristics of Floating Media in MBBR and IFAS Fixed-Film Systems*. Report U4R11, Water Environment Research Foundation (WERF), Alexandria, VA, USA.

Morgenroth, E., van Loosdrecht, M. C. M. & Wanner, O. 2000 Biofilm models for the practitioner. *Water Sci. Technol.* **4** (4–5), 509–512.

Nicolella, C., van Loosdrecht, M. C. M. & Heijnen, J. J. 2000 Wastewater treatment with particulate biofilm reactors. *J. Biotechnol.* **80** (1), 1–33.

Odegaard, H., Christensson, M. & Sorensen, K. K. 2014 Hybrid systems. In: *Activated Sludge – 100 Years and Counting* (D. Jenkins & J. Wanner, eds). IWA Publishing, London.

Okabe, S., Satoh, H. & Watanabe, Y. 1999 In situ analysis of nitrifying biofilms as determined by in situ hybridization and the use of microelectrodes. *Appl. Environ. Microbiol.* **65** (7), 3182–3191.

Pellicer-Nàcher, C., Domingo-Félez, C., Mutlu, A. G. & Smets, B. F. 2013a Critical assessment of methods for the extraction of extracellular polymeric substances from mixed culture biomass. *Water Res.* doi: 10.1016/j.watres.2013.06.026.

Pellicer-Nàcher, C., Franck, S., Gülay, A., Ruscalleda, M., Terada, A. & Smets, B. F. 2013b Sequentially aerated membrane biofilm reactors for autotrophic nitrogen removal: microbial community composition and dynamics. *Microb. Biotechnol.* doi: 10.1111/1751-7915.12079.

Picioreanu, C., Kreft, J.-U. & van Loosdrecht, M. C. M. 2004 Particle-based multidimensional multispecies biofilm model. *Appl. Environ. Microbiol.* **70** (5), 3024–3040.

Picioreanu, C., Head, I. M., Katuri, K. P., van Loosdrecht, M. C. M. & Scott, K. 2007 A computational model for biofilm-based microbial fuel cells. *Water Res.* **41**, 2921–2940.

Pronk, M., de Kreuk, M. K., de Bruin, B., Kamminga, P., Kleerebezem, R. & van Loosdrecht, M. C. M. 2015 Full scale performance of the aerobic granular sludge process for sewage treatment. *Water Res.* **84**, 207–217.

Radu, A. I., Bergwerff, L., van Loosdrecht, M. C. M. & Picioreanu, C. 2015 Combined biofouling and scaling in membrane feed channels: a new modeling approach. *Biofouling* **31** (1), 83–100.

Rittmann, B. E. 2006 The membrane biofilm reactor: the natural partnership of membranes and biofilm. *Water Sci. Technol.* **53** (3), 219–225.

Roeselers, G., van Loosdrecht, M. C. M. & Muyzer, G. 2008 Phototrophic biofilms and their potential applications. *J. Appl. Phycol.* **20**, 227–235.

Rusten, B., Hem, L. J. & Odegaard, H. 1995 Nitrification of municipal wastewater in moving-bed biofilm reactors. *Water Environ. Res.* **67**, 65–74.

Rusten, B., Eikebrokk, B. & Lygren, E. 2006 Design and operations of the Kaldnes moving bed biofilm reactors. *Aquacult. Eng.* **34** (3), 322–331.

Sabba, F., Picioreanu, C., Boltz, J. P. & Nerenberg, R. 2017 Predicting N_2O emissions from nitrifying and denitrifying biofilms: a modelling study. *Water Sci. Technol.* **75** (3), 530–538.

Sandt, C., Smith-Palmer, T., Pink, J., Brennan, L. & Pink, D. 2007 Confocal Raman microspectroscopy as a tool for studying the chemical heterogeneities of biofilm *in situ*. *J. Appl. Microbiol.* **103**, 1808–1820.

Shrout, J. D., Chopp, D. L., Just, C. L., Hentzer, M., Givskov, M. & Parsek, M. R. 2006 The impact of quorum sensing and swarming motility on *Pseudomonas aeruginosa* biofilm formation is nutritionally conditional. *Mol. Microbiol.* **62** (5), 1264–1277.

Srey, S., Jahid, I. K. & Ha, S.-D. 2013 Biofilm formation in food industries: a food safety concern. *Food Control* **31** (2), 572–585.

Syron, E. & Casey, E. 2008 Membrane aerated biofilms for high rate biotreatment: performance appraisal, engineering principles, scale-up and development requirement. *Environ. Sci. Technol.* **42** (6), 1833–1844.

Terada, A., Lackner, S., Kristensen, K. & Smets, B. F. 2010 Inoculum effects on community composition and nitritation performance of autotrophic nitrifying counter-diffusion biofilm reactors. *Environ. Microbiol.* **12**, 2858–2872.

Torresi, E., Fowler, J., Polesel, F., Bester, K., Andersen, H. R., Smets, B. F., Plósz, B., G. & Christensson, M. 2016 Biofilm thickness influences biodiversity in nitrifying MBBRs – implications on micropollutant removal. *Environ. Sci. Technol.* **51**, 9279–9288.

Van der Star, W. R. L., Abma, W. R., Blommers, D., Mulder, J.-W., Tokutomi, T., Strous, M., Picioreanu, C. & van Loosdrecht, M. C. M. 2007 Startup of reactors for anoxic ammonium oxidation: experiences from the first full-scale anammox reactor in Rotterdam. *Water Res.* **41**, 4149–4163.

Van Hulle, S., Callens, J., Mampaey, K., van Loosdrecht, M. & Volcke, E. 2012 N_2O and NO emissions during autotrophic nitrogen removal in a granular sludge reactor: a simulation study. *Environ. Technol.* **33** (20), 2281–2290.

Veuillet, F., Lacroix, S., Bausseron, A., Gonidec, E., Ochoa, J., Christensson, M. & Lemaire, R. 2014 Integrated fixed-film activated sludge ANITA-Mox process – a new perspective for advanced nitrogen removal. *Water Sci. Technol.* **69** (5), 915–922.

Vlaeminck, E., Terada, A., Smets, B. F., De Clippeleir, H., Schaubroeck, T., Bolca, S., Demeestere, L., Mast, J., Carballa, M., Boon, N. & Verstraete, W. 2010 Aggregate size and architecture determine biomass activity for one-stage partial nitritation and anammox. *Appl. Environ. Microbiol.* **76**, 900–909.

Vrouwenvelder, J. S., Graf von der Schulenburg, D. A., Kruithof, J. C., Johns, M. L. & van Loosdrecht, M. C. M. 2009 Biofouling a spiral-wound nanofiltration and reverse osmosis membranes: a feed spacer problem. *Water Res.* **43** (3), 583–594.

Vrouwenvelder, J. S., van Loosdrecht, M. C. M. & Kruithof, J. C. 2011 Novel scenario for biofouling control of spiral wound membrane systems. *Water Res.* **45** (15), 3890–3898.

Wagner, M., Taherzadeh, D., Haisch, C. & Horn, H. 2010 Investigation of the mesoscale structure and volumetric features of biofilms using optical coherence tomography. *Biotechnol. Bioeng.* **107** (5), 844–853.

Wang, J., Lu, H., Chen, G.-H., Lau, G. N., Tsang, W. L. & van Loosdrecht, M. C. M. 2009 A novel sulphate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment. *Water Res.* **43**, 2363–2372.

Wanner, O., Eberl, H., Morgenroth, E., Noguera, D., Picioreanu, C., Rittmann, B. & van Loosdrecht, M. C. M. 2006 *Mathematical Modelling of Biofilms*. Scientific and technical report no. 18. IWA Publishing, London.

Wett, B. 2007 Development and implementation of a robust deammonification process. *Water Sci. Technol.* **56** (7), 81–88. doi: 10.2166/wst.2007.611.

Wolf, G., Picioreanu, C. & van Loosdrecht, M. C. M. 2007 Kinetic modelling of phototrophic biofilms: the PHOBIA model. *Biotechnol. Bioeng* **97** (5), 1064–1079.

Yeon, K.-M., Cheong, W.-S., Oh, H.-S., Lee, W.-N., Hwang, B.-K., Lee, C.-H., Beyenal, H. & Lewandowski, Z. 2009 Quorum sensing: a new biofouling control paradigm in a membrane bioreactor for advanced wastewater treatment. *Environ. Sci. Technol.* **43** (2), 380–385.

Zetterström, R. 2009 The 1998 Nobel Prize – discovery of the role of nitric oxide as a signaling molecule. *Acta Paediatr.* **98** (3), 593–599.

Zhou, M., Yang, J., Wang, H., Jin, T., Xu, D. & Gu, T. 2013 Microbial fuel cells and microbial electrolysis cells for the production of bioelectricity and biomaterials. *Environ. Technol.* **34** (13–14), 1915–1928.

First received 24 July 2016; accepted in revised form 19 January 2017. Available online 2 February 2017