Recent progress using membrane aerated biofilm reactors for wastewater treatment

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ABSTRACT

The membrane biofilm reactor (MBfR), which is based on the counter diffusion of the electron donors and acceptors into the biofilm, represents a novel technology for wastewater treatment. When process air or oxygen is supplied, the MBfR is known as the membrane aerated biofilm reactor (MABR), which has high oxygen transfer rate and efficiency, promoting microbial growth and activity within the biofilm. Over the past few decades, lab-scale studies have helped researchers and practitioners understand the relevance of influencing factors and biological transformations in MABRs. In recent years, pilot- to full-scale installations are increasing along with process modeling. The resulting accumulated knowledge has greatly improved understanding of the counter-diffusional biological process, with new challenges and opportunities arising. Therefore, it is crucial to provide new insights by conducting this review. This paper reviews wastewater treatment advancements using MABR technology, including design and operational considerations, microbial community ecology, and process modeling. Treatment performance of pilot- to full-scale MABRs for process intensification in existing facilities is assessed. This paper also reviews other emerging applications of MABRs, including sulfur recovery, industrial wastewater, and xenobiotics bioremediation, space-based wastewater treatment, and autotrophic nitrogen removal. In conclusion, commercial applications demonstrate that MABR technology is beneficial for pollutants (COD, N, P, xenobiotics) removal, resource recovery (e.g., sulfur), and N2O mitigation. Further research is needed to increase packing density while retaining efficient external mass transfer, understand the microbial interactions occurring, address existing assumptions to improve process modeling and control, and optimize the operational conditions with site-specific considerations.

Key words: commercial application, counter-diffusional biofilm, membrane aerated biofilm reactor (MABR), modeling, nutrient removal, wastewater treatment

HIGHLIGHTS

- Performance of commercial MABR applications is assessed.
- Design and operational considerations are evaluated for both pure and hybrid MABRs.
- Microbial community ecology in the counter-diffusional membrane-aerated biofilm is reviewed.
- Review covers current state-of-the-art process modeling for MABR studies.
- Existing challenges are identified to assist with framing future opportunities for MABR technology.

ABBREVIATIONS

| Abbreviation | Definition |
|--------------|------------|
| ACN          | Acetonitrile                                    |
| AI           | Artificial intelligence                         |
| AOA          | Ammonia-oxidizing archaea                       |
| AOB          | Ammonia-oxidizing bacteria                       |
| AS           | Activated sludge                                |
| BOD          | Biological oxygen demand                        |
| BNR          | Biological nutrient removal                      |
| COD          | Chemical oxygen demand                          |
| Comammox     | Complete ammonia oxidation                       |
| CoMANDR      | Counter-diffusion membrane aerated nitrifying and denitrifying reactor |
| DNA          | Deoxyribonucleic acid                            |
| DO           | Dissolved oxygen                                |

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DOPA  L-3,4-dihydroxyphenylalanine
DPAO  Denitrifying polyphosphate-accumulating organism
EBPR  Enhanced biological phosphorus removal
EPS   Extracellular polymeric substance
FA    Free ammonia
FISH  Fluorescence in situ hybridization
HRT   Hydraulic residence time
IWA   International Water Association
LCA   Life cycle analysis
MABR  Membrane aerated biofilm reactor
MAB   Membrane aerated biofilm
MBBR  Moving bed biofilm bioreactor
MBfR  Membrane biofilm reactor
MBR   Membrane bioreactor
MPC   Model predictive control
mRNA  Messenger ribonucleic acid
N     Nitrogen
NOB   Nitrite-oxidizing bacteria
NR    Nitrification rate
OTE   Oxygen transfer efficiency
OTR   Oxygen transfer rate
OCT   Optical coherence tomography
P     Phosphorus
PAO   Polyphosphate-accumulating organism
PDMS  Polydimethylsiloxane
PHA   Polyhydroxy-alkanoates
PMP   Polymethylpentene
PN/A  Partial nitritation/anammox
PP    Polypropylene
PVDF  Polyvinylidene fluoride
qPCR  Quantitative polymerase chain reaction
rRNA  Ribosomal ribonucleic acid
SI    Supporting information
SIP   Stable isotopic probing
SND   Simultaneous nitrification and denitrification
SOB   Sulfide-oxidizing bacteria
SRT   Solid residence time
TN    Total nitrogen
TOC   Total organic carbon
TP    Total phosphorus
VFA   Volatile fatty acid
VOC   Volatile organic compound
WRRF  Water resource recovery facility
WWTP  Wastewater treatment plant
1D    One-dimensional
2D    Two-dimensional

1. INTRODUCTION

The membrane biofilm reactor (MBfR), based on the counter-diffusions of the electron donor and acceptor, is a promising technology for water and wastewater treatment (Aybar et al. 2012; Martin & Nerenberg 2012; Nerenberg 2016). An MBfR uses a gas-permeable membrane to support a counter-diffusional biofilm where gaseous substrates enter the biofilm opposite to substrates from the bulk liquid (Martin et al. 2013). As the constituents in the gas stream diffuse through the membrane while other substrates diffuse through the liquid boundary layer, biological activity is the highest in the center of the MBfR biofilm, where both gaseous and liquid substrates are sufficient. This counter-diffusion property of MBfRs ensures high functional stability against shock loads and toxic inhibitors (Martin & Nerenberg 2012; Janczewski & Trusek-Holownia 2016; Nerenberg 2016).

The nature of the gaseous substrate is selected to enable the desired biochemical reactions in the MBfR. When air or oxygen is supplied in the lumen, MBfRs are often known as membrane aerated-biofilm reactors (MABRs). In MABRs, the oxygen...
supply in the lumen can be manipulated to create an inner oxygen-rich zone and outer oxygen-depleted zone in the biofilm, housing diverse ecological niches for a range of microbial functions in the same biofilm. The advantages of MABR technology include high effluent quality (Sunner et al. 2018; Sathyamoorthy et al. 2019), high carbon processing efficiency (Houweling & Daigger 2019; Mehrabi et al. 2020), up to 100% oxygen transfer efficiency (OTE) (Heffernan et al. 2017; Bicudo et al. 2019), and compact reactor footprints (Sunner et al. 2018). Compared to other biofilm reactors, like Moving Bed Biofilm Reactor (MBBR) with a typical designed nitrification rate (NR) of 0.5 g N/m²-d, MABRs achieve greatly improved NRs of 1.0–3.0 g N/m²-d (Côté et al. 2015; Kunetz et al. 2016; Peeters et al. 2017a, 2017b; Underwood et al. 2018; Nathan et al. 2020). The greatly improved NRs result from high oxygen transfer rates (OTRs) in MABRs. Moreover, the nitrification performance in MABRs is less susceptible to carbon loadings because the nitrifying population inhabits in the inner biofilm layer (Houweling & Daigger 2019).

Despite the recognized advantages, MABR technology is still in its early stage of commercialization, with developments ongoing to tackle unsolved issues. Lab-scale studies over the last several decades have explored the mass transfer mechanisms, biofilm characteristics, influencing factors, and treatment performance of MABRs, and several reviews have summarized the lessons learned from lab-scale investigations (Casey et al. 1999; Syron & Casey 2008a; Aybar et al. 2012; Martin & Nerenberg 2012; Nerenberg 2016). However, those early reviews did not cover pilot- to full-scale experiences because MABR technology has become commercially available only recently. Commercial interest in MABRs has also grown increasingly since the launch of the first generation of commercial MABR products (Martin et al. 2017; Underwood et al. 2018; Guglielmi et al. 2020). With three commercial MABR products available, i.e. ZeeLung MABR by Suez Water Technologies & Solutions, OxyMem MABR by OxyMem Limited, and Fluence MABR by Fluence Corporation, global practitioners and researchers have significantly improved their understanding of the counter-diffusional biological process from the growing number of the pilot- to full-scale installations. Lu et al. (2020) reviewed several pilots and full-scale MABR applications for municipal and industrial wastewater treatment, but more commercial applications are occurring for different types of wastewater treatment, and long-term operation has been investigated (Guglielmi et al. 2020; Nathan et al. 2020; Uri-Carreno et al. 2021). This paper reviews the further accumulated knowledge and recent advances from the growing commercial installations. Moreover, current lab-scale research is moving towards a deeper understanding of membrane-aerated biofilms (MABs), including the metabolic pathways (Tian et al. 2019; Tian et al. 2020), MAB formation (Hu et al. 2020), predation activities (Aybar et al. 2019; Kim et al. 2020), and novel microbial compositions (Zhang et al. 2021). Process modeling is also improving to facilitate MABR design and operation (Chen et al. 2020; Carlson et al. 2021). However, active biofilm management is still a challenge, and fundamental knowledge is still limited regarding complex interactions between attached and suspended growth. Understanding their unique benefits and existing barriers to broader implementation is essential to set the stage for wider adoption of MABR technology. Future research directions need to be identified to accelerate MABR technology development. Accordingly, the objective of this review is to report and discuss the recent progress using MABRs for wastewater treatment, including:

1. Design and operational considerations, focusing the pilot and full-scale applications. This review also explicitly discussed differences and tradeoffs of hybrid and pure biofilm MABRs;
2. Microbial community ecology in MABRs;
3. Modeling efforts;
4. Performance assessment, including organic carbon, nitrogen (N), and phosphorus (P), removal, xenobiotics treatment, and sulfur recovery;
5. Current challenges and outlook.

This review is also supplemented by a workshop explicitly focusing on MABR technology held at the International Water Association (IWA) Biofilms Virtual Conference 2020, where the authors collected practice-based knowledge and integrated information from the three MABR vendors and a wide range of experts in the field.

2. DESIGN AND OPERATIONAL CONSIDERATIONS

Design and operational conditions impact hydrodynamics and nutrient availability, which then influence mass transfer in MABRs. Design and operational conditions also influence biofilm characteristics, including thickness, density, microbial composition, and kinetics, which impact internal mass transfer and biodegradation rates within the biofilm. In hybrid MABRs, where MABRs are inserted into suspended growth bioreactors, operational conditions like solid retention time
(SRT) also affect microbial activities in the suspended growth. The biochemical reaction rates at which target pollutants are consumed by microorganisms in both the attached and suspended growth define the overall performance of MABR processes (Syron & Casey 2008a). Membrane module configuration, membrane material selection, oxygen transfer and aeration mode, biofilm thickness control, and external mass transfer are among the most critical considerations for MABR design, startup and operation.

2.1. Membrane module configuration and process layout

Hollow-fiber and flat sheet are two common membrane configurations used in MABRs, and both are commercially available (Syron & Heffernan 2017; Shechter et al. 2020a, 2020b). In practice, hundreds of hollow-fiber membranes are potted to create a module, and modules are installed into cassettes for deployment in bioreactors (Figure 1(a)). ZeeLung MABR and OxyMem MABR have adopted membrane cassettes, while the Fluence MABR provides a flat sheet system in which a membrane sleeve is spiral wound around a core, and the liquid flow follows the pattern of an airlift, rising through the spiral spacings and flowing downwards through the core (Figure 1(c)). In both designs, oxygen enters the biofilm from the lumen and is consumed. At the same time, liquid substrates diffuse into the biofilm from the boundary layer with their concentrations decreasing towards the inner biofilm (Figure 1(b)) (Pellicer-Nàcher et al. 2013; Tan et al. 2014; Janczewski & Trusek-Holownia 2016; Lu et al. 2020).

Liquid flow directions can be co-current (Pellicer-Nàcher et al. 2013; Castrillo et al. 2019), counter-current (Christenson et al. 2018), or cross-current (Kunetz et al. 2016) with respect to the inlet gas flow (Figure 2). A lab-scale study compared the oxygen transfer mechanism in co-current and counter-current MABRs (Perez-Calleja et al. 2017). A more significant oxygen partial pressure at the distal end was observed in the counter-current MABR rather than the co-current MABR. The effects of the oxygen partial pressure drop on the system performance will be discussed in the section 2.3 in more detail. However, for the pilot- to full-scale applications, due to the modest depletion of oxygen, greater flow velocity, and well-mixed conditions in bioreactors, impacts of liquid flow directions are generally modest at best.

The MABR unit can either be operated as a pure biofilm process or coupled with conventional activated sludge (AS) as a hybrid biofilm process (Daigger 2020; Carlson et al. 2021). A fundamental difference between the two processes is the location where the organic carbon is metabolized (Carlson et al. 2021). In pure biofilm processes, organic carbon is utilized in the outer biofilm layer adjacent to the bulk liquid by heterotrophic organisms, and nitrification occurs in the inner portion of the MAB (Syron et al. 2015; Kunetz et al. 2016; Bicudo et al. 2019). In Hybrid MABR/AS processes, the organic carbon is mainly utilized by the suspended growth, and a nitrifying biofilm is developed in the attached growth for ammonia removal (Houweling et al. 2018; Shechter & Dagai 2018). The Hybrid MABR/AS process represents a full-scale solution for upgrading existing water resource recovery facilities. In practice, MABR units are commonly submerged in anoxic tanks to increase the

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**Figure 1** | Schematic diagram of the MABR and concentration profiles of limiting substrates. (a) Cassette and module of the hollow-fiber MABRs. (b) Concentration profiles in MABRs. (c) Spiral wound of the flat sheet MABRs. Grey arrows indicate the liquid flow pattern.
biomass inventory, intensify the conventional AS process, and increase the facility's treatment capacity (Figure 3(a)). With the aerobic MAB present in the anoxic zone, the Hybrid MABR/AS process can facilitate simultaneous nitrification and denitrification (SND) in a single tank and eliminate the internal nitrate recycling (Carlson et al. 2021). However, in such anoxic Hybrid MABR/AS system, a modest amount of oxygen will be transferred into the anoxic zone when air scouring is applied for biofilm thickness control, which could affect denitrification activities.

Researchers also investigated the feasibility of incorporating MABR units into the aerobic zone with internal nitrate recycling (Figure 3(b)) (Sun et al. 2020). With the aerobic MAB present in the aerobic zone, some bacteria capable of heterotrophic nitrification-aerobic denitrification, such as Thauera and Paracoccus, may facilitate the SND mechanism (Sun et al. 2020). For enhanced biological phosphorus removal (EBPR) systems, an initial anaerobic zone can be added upstream for hydrolysis of particulate and colloidal substrates and volatile fatty acids (VFAs) uptake by polyphosphate-accumulating organisms (PAOs). Incorporation of MABR units into an anaerobic zone can be problematic because membrane aeration introduces electron acceptors, i.e., dissolved oxygen (DO) directly and also nitrate if nitrification occurs, that reduces fermentation and allows other heterotrophs to compete with PAOs for VFAs that may be present. Placement of the MABR in the anoxic tank is an established practice for biological nitrogen removal (Houweling et al. 2017; Underwood 2017).
et al. 2018; Guglielmi et al. 2020). More research and comparative studies are needed to analyze the ecological niches created and treatment performance achieved when MABRs are placed in anaerobic, anoxic, or aerobic zones in hybrid processes.

Packing density is a critical parameter to size the MABR zone in the hybrid process. While a high packing density can provide a larger specific surface area to support biofilm attachment and consequently promote the pollutant removal rates, biofilm bridging can occur at high packing densities, resulting in a decrease in the effective surface area (Hou et al. 2019). Research and practice experiences are in need to investigate solutions to increase packing density while retaining efficient external mass transfer characteristics and avoiding solids build-up. This will lead to smaller units and further reductions in footprints and cost.

2.2. Membrane material selection

Membrane properties, including surface morphology, porosity, and permeability, have a significant impact on MABR performance as they affect microbial affinity for the membrane and oxygen transfer, which ultimately impact biofilm characteristics and biochemical reaction rates (Syron & Casey 2008a; Lu et al. 2020). Improper membrane materials can result in low oxygen transfer rates and poor biomass adhesion, hampering MABR performance with a longer startup phase. Membranes in MABRs are typically composed of hydrophobic materials such as polyvinylidene fluoride (PVDF) and polypropylene (PP) to enhance microbial attachment to form a functional biofilm (Hou et al. 2013; Lu et al. 2020; Xiao et al. 2021). A comparative study between PVDF and PP membranes showed that the PVDF membrane exhibited better resistance to pore blocking issues and improved biomass attachment to the MABR because of its higher hydrophilicity and surface roughness (Wu et al. 2019). Nylon silk was also used as the membrane material for surface water treatment (Janczewski & Trusek-Holownia 2016). These membrane materials are generally favored in lab-scale MABR studies because high oxygen transfer flux through pores promotes aerobic biochemical reaction rates (Syron & Casey 2008a; Nerenberg 2016; Lu et al. 2020). However, microporous membrane materials are not used in the pilot- or full-scale applications due to pore-clogging issues, low life span, and low air pressure requirements.

In contrast, dense membrane materials are used in commercial MABR units because of their increased strength, durability and decreased likelihood of membrane fouling (Syron & Casey 2008a; Nerenberg 2016; Lu et al. 2020). Examples of dense membrane materials include polymethylpentene (PMP) and silicone polydimethylsiloxane (PDMS). For instance, OxyMem uses PDMS membranes, with benefits including high oxygen permeability, chemical resistance, and resistance to mechanical stress (Bicudo et al. 2019).

A composite membrane combines a dense layer coated with a microporous layer. Hou et al. (2013) reported that a L-3,4-dihydroxyphenylalanine (DOPA) coated PVDF membrane increased the gas flux by two times and shortened the time required to achieve an optimal chemical oxygen demand (COD) removal rate from 6 hours to 0.5 hours. Overall, experience with composite membranes or surface modifications is still limited. Further research is needed to develop high-efficient and low-cost composite membrane materials for wastewater treatment using MABRs.

2.3. Oxygen transfer and aeration modes

OTRs and OTEs are two key performance indicators used to evaluate MABR processes (Houweling et al. 2017). The OTR across the membrane can be described as (Pellicer-Nàcher et al. 2013):

\[
J_{O_2} = K \left( \frac{S_{O_2,g}}{H} - S_{O_2,\text{bio}} \right)
\]

where \( J_{O_2} \) is the OTR across the membrane (g/m²·d), \( K \) is the mass transfer coefficient (m/d), \( S_{O_2,g} \) and \( S_{O_2,\text{bio}} \) are the oxygen concentrations in the gas phase and membrane-biofilm interface (g/m³), and \( H \) represents the unitless Henry's Law constant. The OTE is characterized as (Houweling & Daigger 2019):

\[
OTE = \frac{X_{O_2,\text{in}} - F_VX_{O_2,\text{out}}}{X_{O_2,\text{in}}}
\]
where \( X_{O_2,\text{in}} \) and \( X_{O_2,\text{out}} \) are the mole fractions of oxygen in the air inlet and outlet (unitless); \( F_v \) is the unitless volumetric loss factor of oxygen:

\[
F_v = \frac{1 - X_{O_2,\text{in}}}{1 - X_{O_2,\text{out}}}
\]

(3)

MABR aeration modes can be categorized as either flow-through or dead-end, according to the bulk gas flow. Higher average OTRs, which translate into higher average contaminant removal fluxes and treatment capacity, are usually achieved in the flow-through rather than dead-end operation (Syron & Casey 2008b). Therefore, the flow-through operation is preferred in commercial applications (Kunetz et al. 2016; Peeters et al. 2017a, 2017b; Underwood et al. 2018; Guglielmi et al. 2020). The higher average OTRs in flow-through MABRs can be explained by the higher oxygen partial pressure, which allows uniform intra-membrane oxygen velocities in the membrane lumens and relatively constant biofilm thickness. For dead-end operation, all the supplied oxygen can be delivered into the biofilm, leading to a 100% OTE and much reduced aeration energy (Tian et al. 2020). However, the dead-end mode has relatively lower OTRs because of oxygen exhaustion and the back-diffusion of gases which cause significant oxygen partial pressure drop and consequently reduced biological reaction rates (Perez-Calleja et al. 2017). Therefore, it is important to consider the tradeoffs between the OTR vs. OTE and treatment capacity vs. aeration energy when selecting the aeration modes in practice. Innovative strategies are also available to balance such tradeoffs. For example, Perez-Calleja et al. (2017) explored the feasibility of operating MABRs in alternating flow-through and dead-end modes. Experimental and modeling results indicated that the transient behavior shifting between the two aeration modes could improve both OTE and OTR. More research is still needed to optimize the interval of switching and duration of each mode, and the optimization is likely to be unique in response to a range of factors, including oxygen partial pressure, oxygen flow rates, and treatment goals.

It should be noted that aeration control via manipulating the oxygen partial pressure is often not practiced in commercial applications. Instead, oxygen is introduced into the MABR lumen at the design airflow rate, and oxygen partial pressure in the lumen changes as a result of oxygen transfer and microbial consumption (Côté et al. 2015; Bicudo et al. 2019). For Zeelung and OxyMem MABRs, this is done because exhaust gas from the MABR is used to create fluid flow through the MABR bundle to renew the fluid inside the bundle and to increase the mass transfer of substrates from the bulk liquid into the biofilm on the MABR membranes (Downing 2021). A summary of aeration parameters used in several commercial MABR applications is provided in Table 1. Reported OTEs were consistently higher than that of the conventional fine bubble aeration system (typically 10%) (Houweling & Daigger 2019). In mainstream commercial applications, a modest proportion of the oxygen in the feed gas (20–30%) is transferred into the biofilm, and the rest is exhausted. As a result, the oxygen partial pressure only declines slightly along the lumen, allowing high OTRs throughout the membrane. As discussed above, high OTRs support higher aerobic biological activities in the MAB, such as nitrification (Houweling & Daigger 2019). Such operations can maximize the ammonia removal fluxes and minimize the membranes needed, and therefore it is an economical choice to sacrifice aeration energy to achieve higher treatment capacity. However, even though the tradeoff between treatment capacity vs. aeration efficiency exists, reported aeration efficiencies of MABRs ranging from 4 to 14 kg O₂/kWh (Table 1) were consistently superior to that of conventional wastewater aeration systems of 1.0–1.5 kg O₂/kWh (Rosso et al. 2008). As aeration accounts for the most considerable energy cost in wastewater treatment plants (WWTPs) (Longo et al. 2016), the bubble-less aeration technology in MABRs, with demonstrated high aeration efficiency, can lead to significant energy savings.

Using pure oxygen instead of air in MABRs enhances OTRs and oxygen penetration depth (Brindle et al. 1998; Cole et al. 2004), enabling high COD and ammonia removal rates (Syron & Heffernan 2017). When operating with pure oxygen, the MABR process may require up to five times less membrane area than when operating with air, leading to capital investment reduction and a smaller footprint (Syron et al. 2015). However, MABRs fed with pure oxygen tend to grow thicker biofilms and increased mass transfer resistance because of the enhanced growth of aerobes. Several studies also reported that air scouring could not effectively control biofilm thickness in this case, which can limit the overall removal performance of MABRs (Stricker et al. 2011; Syron et al. 2015). Moreover, excessive oxygen may create ecological niches that suppress nirS and nirK genes for denitrification (Cole et al. 2004). The choice of air or pure oxygen depends on the wastewater characteristics, treatment goal, and operational considerations. For example, feeding air may not be sufficient to treat wastewater with high organic carbon loadings, and in this case, pure oxygen may be beneficial to improve COD removal efficiency if the biofilm is not biomass limited. Ongoing research and practical experience are required to elucidate further the effects
of pure oxygen on biofilm characteristics, including density, thickness, and microbial composition. Effective control strategies are needed to control the potentially thicker biofilms grown with pure oxygen feed.

Flow-through and dead-end aeration can both be operated in continuous or intermittent mode. While continuous aeration is typically applied for COD and nutrient removal processes, intermittent aeration is commonly selected when an oxygen limiting condition is desired to enrich ammonia-oxidizing bacteria (AOB) and suppress nitrite-oxidizing bacteria (NOB) for shortcut ammonia removal in MABR biofilms (Pellicer-Nàcher et al. 2014; Ma et al. 2017a, 2017b; Bunse et al. 2020). In a pilot-scale MABR operated for side-stream shortcut ammonia removal, i.e., the ZeeNAMMOX process, the vertical oxygen supply direction in the lumen was alternated periodically to switch the DO limited condition (oxygen <2%) from top to bottom (Long et al. 2020). This study applied exhaust gas-based aeration control, which used oxygen sensors to measure the oxygen content in the

| Wastewater type and application purpose | Scale | Air feed pressure (MPa) | Airflow rate (Normal L/m²-h) | Feed gas | Aeration mode | OTR (g O₂/m²-d) | OTE (%) | Aeration efficiency (kg O₂/kWh) | References |
|----------------------------------------|-------|------------------------|-------------------------------|----------|---------------|----------------|---------|-------------------------------|------------|
| Municipal wastewater for nitrogen and phosphorus removal | Pilot | 0.067 | 4.3 | Process air | Continuous flow-through | 8–12 | NA | 6.5–7.0 | Kunetz et al. (2016) |
| Municipal wastewater for N removal | Pilot | 0.067 | 5.3 | Process air | Continuous flow-through | 8–15 | 30–40 | 6 | Côté et al. (2015) |
| Municipal wastewater for COD and nitrogen removal | Pilot | NA | NA | Process air | Continuous flow-through | NA | NA | 4.0–4.9 | Sunner et al. (2018) |
| Municipal wastewater for COD and nitrogen removal | Full | 0.048 | 4.8 | Process air | Continuous flow-through | 7.1–15.9 | 25, up to 50 | NA | Guglielmi et al. (2020) |
| Municipal wastewater for nitrogen and phosphorus removal | Pilot | 0.025 | NA | Process air | Continuous flow-through | Average: 1.5–4.5 Peak: 9 | 25–50 | 14 | Bicudo et al. (2019) |
| Industrial wastewater for COD and nitrogen removal | Pilot | 0.041 | 1.8 | Process air | Continuous flow-through | 3 | 25 | NA | Stricker et al. (2011) |
| Industrial wastewater for COD and nitrogen removal | Pilot | 0.041 | 1.4 | Process air | Continuous flow-through | 2.9 | 31 | NA | Stricker et al. (2011) |
| Landfill leachate for nitrification | Pilot | 0.025 | NA | Process air or pure oxygen | Continuous flow-through | 8 with process air, 25 with pure oxygen | 20–75 with process air; 50–80 with pure oxygen | 4 | Syron et al. (2015) |
off-gas. The measured oxygen content was then correlated to ammonia removal and residual ammonia concentrations, and therefore, the use of ammonia sensors was eliminated. Reliable suppression of NOB growth was achieved, limiting the ratio of nitrate generation to ammonia oxidation to 0.2. The alternation of oxygen feed direction also exposed the biofilm to more uniform conditions along the length of the membranes. Gas from the outlet is usually not recycled due to (1) the depletion of oxygen in the lumen phase which, when recycled, would decrease the OTR (Uri-Carreno et al. 2021), and (2) back-diffusion can dilute the oxygen concentration in the lumen (Perez-Calleja et al. 2017).

It is also critical to consider the tradeoff between nitrification and total nitrogen (TN) removal under different aeration conditions. A higher oxygen availability can boost nitrification but also limit denitrification activity, reducing TN removal rates (Mehrabi et al. 2021). In contrast, operating at a lower air pressure or airflow rate can limit oxygen availability and promote TN removal in the MABR. In addition, the high availability of oxygen may lead to excessive growth of aerobes and thereby thick biofilms that are more prone to mass transfer limitations (Shanahan & Semmens 2015). Therefore, a tradeoff exists between nitrification and TN removal under different aeration conditions.

2.4. Biofilm thickness control

Biofilm thickness control is an important consideration for MABR operation, as excessive biofilm thickness, which is a frequently encountered problem in practice, raises concerns for increased mass transfer resistance and membrane clogging issues. In practice, biofilm thickness control is commonly accomplished by promoting biofilm detachment, which involves introducing scouring air bubbles onto the biofilm surface to remove the external layer of the biofilm (Heffernan et al. 2017). Commercial MABRs collect the exhaust gas for air scouring (Nathan et al. 2020; Downing 2021). Specifically, the OxyMem MABR has a unique scouring system that uses inert gas and a pressure decay test to measure the relative biofilm thickness, which controls a scouring system that intermittently creates air bubbles to encourage biofilm detachment (Casey et al. 2014).

For pure biofilm systems where both ammonia and organic carbon are metabolized in the attached growth, biofilm thickness control is critical to reduce mass transfer resistance. NRs in the MAB were doubled after air scouring because of the improved mass transfer rates of ammonia (Supporting information (SI)). However, if too much biofilm is removed, the attached growth may not retain sufficient functional biomass, diminishing performance. Several studies have reported performance upsets following biofilm detachment events due to the reduced number of organisms in the MAB, and the unwanted performance lag can last up to several months before full efficiency was recovered (Shanahan et al. 2005; Bunse et al. 2020). More research is needed to investigate how a biofilm responds to scouring events, including detachment/reattachment rates, microbial composition shifts, and changes to the physical properties of the biofilm. Practical experience is critical to identify the optimal air scouring frequency and intensity for biofilm detachment to help reduce the performance lag observed after air scouring events.

The MAB in hybrid systems behaves differently than that in pure biofilm systems. The MAB thickness in hybrid systems is often thinner, and less biofilm control is generally needed. This occurs because the suspended growth in the bulk liquid consumes soluble COD, thereby minimizing heterotrophic growth in the biofilm (Downing et al. 2010; Uri et al. 2018). Biofilm thickness does respond to temperature and operational conditions. For example, thicker biofilms tend to form during winter and short suspended growth SRT operations, while biofilms get thinner during summer and long SRT operations (SI). However, excessive biofilms can develop in Hybrid MABR/AS processes, raising clogging and mass transfer resistance issues because of the decreased effective biofilm surface area. They may also displace bioreactor volume for suspended growth and diminish the treatment performance contributed by the suspended growth (Houweling & Daigger 2019). Therefore, biofilm thickness control via air scouring is often applied in hybrid systems to ensure effective performance. Of course, overuse of air scouring in Hybrid MABR/AS systems must be avoided when the MABR unit is incorporated into the anoxic tank to prevent possible adverse impacts on denitrification. Therefore, understanding how a Hybrid MABR/AS process responds to scouring events is critical to control the biofilm as well as the suspended growth to maximize overall treatment performance.

2.5. External mass transfer

Bulk liquid hydrodynamic conditions affect external mass transfer from the bulk liquid to the biofilm in MABRs (Bicudo et al. 2019). The liquid flow produced by air bubbling renews the substrates in the bulk liquid within the membrane bundles so that continued substrate removal can occur (Daigger 2020). Liquid flow velocity, coupled with the introduction of air bubbles for scouring and promoting fluid flow, creates fluid shear on the biofilm surface. As a result, liquid flow velocity affects the thickness of the boundary layer at the liquid-biofilm interface and the biofilm detachment rate. Diffusion boundary layer thickness
is reduced with increased liquid flow velocity, and biofilm detachment accelerates. As a result, the thinner diffusion boundary layer enhances the external mass transfer rate, providing higher overall removal rates (Shoji et al. 2020). However, in a previous tracer experiment (Castrillo et al. 2019), high flow velocities resulted in a non-optimal flow pattern, allowing no further enhancement of mass transfer. This lack of increase in mass transfer could be caused by flow channeling or short-circuiting, so the liquid flow velocity must be monitored to manage optimal reactor performance.

Liquid flow velocity also impacts MAB structure. Lab-scale studies reported a non-uniform biofilm density along the membrane of biofilms grown at a low fluid velocity (1.7 and 2 cm/s) (Shanahan et al. 2005; Pellicer-Nàcher et al. 2014). This resulted from the variation in the effective local diffusivity, which had a profound effect on biofilm stratification and microbial activities. However, such effects would not be observed for reactor configurations where flow recirculation is used to create the necessary fluid flow velocity, such as the case for some laboratory- and commercial-scale units. In commercial applications, gas sparging is often used to create flow through membrane bundles. The rate of gas sparging will control the liquid flow velocity independent of the organic and nitrogen loading on the membranes. Therefore, the biofilms in commercial MABRs are exposed to relatively constant diffusivity and liquid substrates. The representative vendor should be able to provide information on the effects of liquid flow velocity on performance for their respective units, which depend on the MABR configuration and method for creating liquid flow velocity.

### 3. MICROBIAL COMMUNITY ECOLOGY

Activities of the complex microbial communities that form are the foundation for the biological transformations that occur in MABR systems. Important elements in the microbial community ecology include taxonomic diversity, functional pathways, and interactions between microbial groups (Konopka 2009). A meaningfully defined microbial community ecology is crucial in developing an operational strategy that affects system performance, energy consumption, and costs. To date, taxonomic diversity and functional pathways in MABRs have been studied using a range of technologies. However, knowledge is still limited to systematically understand microbial interactions in the MABR and their shifts in response to operational conditions.

#### 3.1. Taxonomic diversity

The counter-diffusional property of MABs results in distinct microbial community structures and behaviors. In most studies, Proteobacteria is the most abundant phylum present in MABRs (Cole et al. 2004; Kinh et al. 2017a, 2017b; Tian et al. 2017; Li & Liu 2019; Sathyamoorthy et al. 2019). Specifically, sub-Proteobacteria, Alphaproteobacteria, and Gammaproteobacteria, prefer anaerobic environments and inhabit the outer biofilm layers or suspended growth, while Betaproteobacteria and Deltaproteobacteria tend to inhabit the aerated portions within the biofilm (Lan et al. 2018; Sathyamoorthy et al. 2019). Proteobacteria is expected to be a key player responsible for nitrogen removal, and both AOB and NOB guilds have been observed in this phylum (Lan et al. 2018; Baskaran et al. 2020). Excessive growth of the filamentous Alphaproteobacteria (‘Nostocoida-like’) and Gammaproteobacteria (Thiothrix and type 021N) are commonly associated with sludge bulking and foaming (Nielsen et al. 2009).

Abundant Flavobacteria of Bacteroidetes phylum in the MAB were also observed in the lab- and pilot-scale investigations (Aybar et al. 2019; Sathyamoorthy et al. 2019). Bacteroidetes organisms are reported to be responsible for phosphorus removal (Xu et al. 2018). In a pilot-scale MABR system, considerable relative abundances of Actinobacteria in both biofilm (7%) and mixed liquor (14%) were identified (Sathyamoorthy et al. 2019). An early study suggested that Actinobacteria may play an important role in the EBPR process but can also lead to operational problems of bulking and foaming (Seviour et al. 2008). Overall, researchers have identified key categories of organisms performing a variety of functions within the microbial community in MABRs. However, this field still needs more research to fully understand microbial interactions between the microbial groups, e.g., symbiosis, competition, or predation, during the wastewater treatment process. This is critical to translate the observed biodiversity to the operation and management of MABRs.

Higher life forms like eukaryotes are also part of the microbial community in MABs. At the genus level, Aybar et al. (2019) reported a substantial abundance of amoeba and flagellated protozoa in MABs. MAB images showed internal voids due to protozoa predation (Kim et al. 2020). Such voids can alter biofilm properties like porosity, density, and mechanical strength, mass transfer of substrates, and microbial community ecology, all of which ultimately affect the net reaction rate in MABRs (Syron & Casey 2008a). More research is needed to fully characterize the conditions affecting the type and activity of eukaryotes and the effects of predation on biofilm formation, detachment, and microbial dynamics in MABRs.
3.2. Functional pathways for biological nutrient removal (BNR)

MABRs enable SND for novel nitrogen removal and carbon degradation compared to the various conventional nitrogen removal schemes. Nitrification, predominantly carried out by AOBs and NOBs, occurs in the inner-most aerobic MAB layer, while denitrification occurs in the anoxic layer and/or suspended growth as heterotrophic denitifiers occupy the outer layer or bulk liquid (Landes et al. 2021; Zhao et al. 2021). Early MABR studies identified dominant nitrifying populations such as *Nitrosomonas* and *Nitrosospira* in the AOB guild as well as *Nitrospira* and *Nitrobacter* in the NOB guild (Liu et al. 2010; Gilmore et al. 2013). Ammonia-oxidizing archaea (AOA), e.g., *Candidatus Nitrososphaera*, was also identified in the MAB as a functional group for nitrification under low oxygen supply (Liu et al. 2016; Tian et al. 2017).

The denitrifying population was more diverse than the nitrifying population, and the relative dominance varied according to the COD loadings and pH (Tian et al. 2015). Some commonly expressed genes throughout the SND process include ammonia monoxygenase gene (*amoA*) for ammonia oxidation and nitrite reductase genes (*nirK* and *nirS*) for nitrite reduction. Several lab-scale studies have confirmed the coexistence of *amoA*, *nirK*, and *nirS* in the MAB, which validates the SND pathway at the genetic level (Cole et al. 2004; Liu et al. 2015).

Anaerobic ammonia oxidation, or anammox, is another innovative pathway that has been intensively studied in MABRs for nitrogen removal (Gong et al. 2007; Cema et al. 2011; Gilmore et al. 2013; Bunse et al. 2020). Anammox is usually combined with partial nitrification, where ammonia is partially oxidized to nitrite by AOBs, and the remaining ammonia is oxidized to nitrogen gas anaerobically via nitrite reduction by anammox bacteria. NOBs, which compete for nitrite with anammox bacteria, are undesirable in partial nitrification/anammox (PN/A) MABRs, and an oxygen-limited condition is employed to out-select NOBs (Gong et al. 2007; Cema et al. 2011; Cho et al. 2019). Compared to traditional nitrogen removal via nitrification and denitrification, the PN/A process reduces both oxygen and carbon requirements (Kuenen 2008; Cho et al. 2019). Core genes for hydrazine metabolism (*hiz* and *hdh*) and the gene cluster for nitrite reduction (*nirK* and *nirS*) are all essential for anammox (Speth et al. 2016). A previous study detected the anammox species *Candidatus Jettenia*, *Ca. Brocadia*, and *Ca. Anammoxoglobus* in an MAB (Zhao et al. 2021). As strict anaerobic organisms, anammox bacteria grow in the anaerobic outer layer of the MAB or suspended growth, and AOBs grow in the inner aerobic biofilm layer (Gong et al. 2007; Bunse et al. 2020). Proliferation of R-strategist AOBs (e.g., *Nitrosomonas*) in the inner MAB layer can quickly consume DO and mitigate the inhibitory effect of oxygen on anammox growth (Terada et al. 2010). Overall, the successful application of the anammox process for nitrogen removal in MABRs relies on the symbiosis between AOBs and anammox bacteria, which is still challenged by the low growth rate of anammox bacteria and microbial competition by NOBs for the nitrite formed by AOBs.

In terms of phosphorus removal, the same metabolic pathways exist in MABRs as conventional AS approaches. PAOs incorporate VFAs in the anaerobic zone for the production of a stored fat reserve, polyhydroxy-alkanoate (PHA), while intercellular supplies of glycolen and polyphosphate are exhausted for energy. In the subsequent aerobic phase, the stored reserve is oxidized back to glycogen, and PAOs uptake excess phosphate from the mixed liquor to regenerate stored polyphosphate (Arun et al. 1988). Some commonly expressed genes throughout the metabolic processing of phosphorus code for key enzymes including: polyphosphate kinase (*ppk*) which facilitates phosphorylation of the polyphosphate chain, exophosphatase (*ppx*) that catalyzes polyphosphate hydrolysis, and adenylate kinase (*adk*) that regulates energy levels in the cell (Akiyama et al. 1993; Shiba et al. 2000; Kristiansen et al. 2013). These annotated functional gene sequences have been valuable to understand the shared metabolic pathways among different PAO taxonomies, such as *Accumulibacter* and *Tetrasphaera* (Kristiansen et al. 2013), and used as marker genes for fine-scale phylogenetic distribution (Gao et al. 2017).

Phosphate uptake can also be coupled with nitrate or nitrite reduction in the anoxic environment via the denitrifying PAOs (DPAOs) (Díez-Montero et al. 2016). The ability to use nitrate and nitrite as the terminal electron instead of oxygen has been demonstrated for selected *Accumulibacter* lineages (Carvalho et al. 2007; Flowers et al. 2009). In the Hybrid MABR/AS process, the nitrate and nitrite produced by the aerobic MAB could diffuse into the anoxic bulk liquid and serve as the electron acceptor for the DPAO metabolism (Carlson et al. 2021). This can lead to significant energy saving for aeration. However, identification of PAOs and DPAOs and the functional mechanism of phosphorus removal remain understudied areas regarding pure MABRs or Hybrid MABR/AS systems.

3.3. Microbial community characterization tools

Three main approaches have been applied to obtain a holistic view of the functioning microbial world in the MABR process: physical analysis, microscopic imaging, and ‘omic’ techniques. Microsensors are commonly used for physical analysis to measure the concentration of key substrates such as DO, pH, ammonia, nitrite, and nitrate in increments as low as 10 μm
within the biofilm (Gilmore et al. 2009). Profiles of these substrates allow observation of the microbial stratification into aerobic, anoxic, and anaerobic regions of an MAB, and the abundance of bacterial guilds and substrate uptake rates in each region can be calculated (Tan et al. 2014). A comparative study examined emissions of the strong greenhouse gas nitrous oxide (N$_2$O) in MABs and co-current biofilms by measuring DO and N$_2$O throughout the depth of the biofilm via microsensors (Kinh et al. 2017a, 2017b). The results showed for the first time that the MABR can mitigate N$_2$O emissions because the N$_2$O produced by AOBs in the aerobic biofilm layers can be uptake in the adjacent anoxic layers by heterotrophs. Microsensors have been used in a variety of MABR studies for in situ monitoring of ammonia removal (Underwood et al. 2018), SND (Underwood et al. 2018), the occurrence of sulfate reduction (Tan et al. 2014), and DO profiles (Aybar et al. 2019). The application of microsensors has revealed that exposure to sublethal concentrations of toxins caused a significant increase in oxygen and hydrogen ion flux from the bulk liquid to the MAB (McLamore et al. 2010). This result linked cellular stress response to bulk liquid water quality, which is critical to establishing effective monitoring and control strategies in MABRs.

Microscopic methods can be extremely helpful in visualizing the mesoscale structure and volumetric features of biofilms. Fluorescence in situ hybridization (FISH) uses a fluorescent-labeled probe that attaches to specific genomic regions of a microorganism, with fluorescence observed under a confocal laser scanning microscope to see the microorganism’s location within the biofilm. FISH is frequently applied on MABR biofilms to identify the presence and spatial distribution of bacterial groups. Nitrosospira and Nitrosonomas have been revealed by FISH as the dominant AOBs in the inner portion of a nitrifying MAB (Liu et al. 2010; Gilmore et al. 2013). Nitrosonomas, known as an R-strategist which favors higher DO concentration than Nitrosospira, was located in the inner-most layer near the membrane surface (Terada et al. 2010). The same studies also observed Nitrospira and Nitrobacter as the dominant NOBs adjacent to the AOB layer.

A separate microscopic technique, optical coherence tomography (OCT), can show the presence of microbial predators within an MAB (Aybar et al. 2019; Kim et al. 2020). However, a major hurdle for the use of microscopic methods in MABRs is sampling, which is invasive and involves cutting the membrane so that the biofilm is exposed. In addition, due to the variations in the biofilm along the MABR fiber, multiple samples need to be analyzed to account for the biofilm heterogeneity. Other microscopic analysis techniques involving Confocal Raman micro-spectroscopy have been used to monitor the live bacterial biofilm as a function of space and time (Sandt et al. 2007). Though very few studies have applied Confocal Raman micro-spectroscopy for biofilm analysis in MABRs, it is a promising non-invasive technique for future studies for the in situ mapping, tracking, and identification of microbes in MABs.

Omics methods, such as deoxyribonucleic acid (DNA) sequencing technologies, provide unprecedented insights into the biodiversity and genetic information of the microbial community in MABRs. This is extremely important to understand the biological transformations of pollutants and behaviors of functional microorganisms in the MABR process. In particular, 16S ribosomal ribonucleic acid (rRNA) gene sequencing, which targets the widely conserved 16S rRNA gene, continues to be one of the most commonly applied methods for taxonomy identification and quantification in both lab-scale MABs and pilot hybrid systems (Kinh et al. 2017a, 2017b; Sathyamoorthy et al. 2019). Quantitative polymerase chain reaction (qPCR) has also been widely used in MABR studies to quantify the presence of a targeted gene and its relative or absolute abundance (Cole et al. 2004). In a recent full-scale Hybrid MABR/AS system, amoA, anammox 16S, Nitrosira 16S, and Nitrobacter 16S rRNA genes were targeted to analyze the nitrifying populations via qPCR analysis (Underwood et al. 2018). Results of the same study indicated a large imbalanced abundance of NOBs and low abundance of AOBs, suggesting the possible existence of the complete ammonia oxidation (comammox) bacteria fully nitrifying ammonia to nitrate.

The downsides of 16S rRNA sequencing and qPCR-based analysis are that they cannot describe the metabolic pathways (Dick 2018). In addition, as the so-called ‘universal’ PCR primers target the 16S rRNA gene of highly conserved sequence and length that can be reliably amplified and compared to a database, 16S rRNA sequencing may miss novel organisms that have genes of unknown sequences. Shotgun metagenomics improves upon the sequencing of targeted genes and provides a means of assessing the total genetic material in a microbial community (Dick 2018). Delgado et al. (2020) examined nitrogen metabolism in an MAB with the presence of sulfide using metagenomic approaches. Results indicated that sulfide could disrupt nitrification by decreasing nitrite oxidation rates but increasing ammonia oxidation rates and N$_2$O production. Hu et al. (2020) used metagenomic sequencing to investigate MAB formation in a phenolic wastewater treatment process. Their results suggested the formation and performance of the biofilm were potentially regulated by quorum sensing systems which can be highly impacted by influent phenolic loadings. Therefore, an optimized phenolic loading rate can be critical to foster MAB formation and shorten the startup time of the system. The metagenomic analysis is also powerful in discovering novel genes and organisms. One of the most recent examples is shown in the discovery of comammox bacteria, which has the
full suite of nitrification genes to oxidize ammonia to nitrate (Daims et al. 2015). Such metagenomic analysis could be used on MABRs in future studies to validate the presence of comammox bacteria and other novel organisms, which, if present, could impact the performance of nitrogen removal.

While metagenomic sequencing provides information on the abundance and metabolic potential of organisms within an MABR, it does not describe whether those processes are active across time or space. In contrast, quantifying messenger ribonucleic acid (mRNA) and proteins with metatranscriptomics and metaproteomics, respectively, can show which genes are actively being expressed and translated into proteins. In addition, stable isotopic probing (SIP) is very powerful to identify cross-feeding, which is the phenomenon of one species living off the metabolic products of another species in complex microbial communities (Mooshammer et al. 2021). Applying those advanced microbial tools in further MABR studies can help quantify the relative contributions of different microbial groups to nutrient removal and microbial interactions between the attached and suspended growth. These omics approaches can be costly and time-intensive, however, and therefore have not been applied to MABR studies to date. The selection of methods should, of course, be based on the scientific questions of interest and research objectives.

4. MODELING

Mathematical modeling is essential to understand and develop biofilm reactors. Given the complex biological treatment configurations of MABRs, mathematical models are frequently used to assist with system design and operation. The ability of models to capture the integrated behavior of complex systems makes them useful research tools, often used in combination with other research techniques, such as molecular approaches discussed in the previous section and wet chemistry analysis.

Conventional biofilm modeling practice (Wanner et al. 2006) and the framework for good biofilm reactor modeling practice (Rittmann et al. 2018) are fully applicable to MABR modeling. However, a unique aspect of MABR modeling is the need to expressly re-formulate oxygen transfer. In conventional co-current biofilms, oxygen is first transferred into the liquid through the gas-liquid interface and then acts like other substrates that diffuse into the biofilm from the liquid (Wanner et al. 2006). As a result, an oxygen-rich zone of active growth develops adjacent to the biofilm-liquid boundary layer. In contrast, in an MAB, oxygen is transferred to the inner surface of the MABR membrane, diffuses through the membrane, and then enters the biofilm. The MAB layer adjacent to the membrane is an oxygen-rich zone of active growth. Given the different mass transfer schemes and distribution of biomass growth, MABs respond to operational and kinetic parameters differently from conventional biofilms (Syron & Casey 2008b). Martin et al. (2017) reported that the diffusivity of ammonia nitrogen, biofilm thickness, and liquid-biofilm boundary layer thickness mostly impacted the NRs in MABs. In contrast, NRs in conventional biofilms are mostly influenced by the liquid-biomass boundary layer thickness, maximum AOB growth rate, and half-saturation constant of oxygen for AOBs. Therefore, MABR models will have to be calibrated differently than traditional biofilm models for a specific application.

MABR modules that account for the unique mass transfer scheme are available in several commercial simulators. This availability allows researchers and practitioners to use models to investigate the impact of process conditions, including bulk biological oxygen demand (BOD) concentrations (Downing & Nerenberg 2008a, 2008b), DO gradients (Downing & Nerenberg 2008a, 2008b), intra-membrane gas pressure (Ahmadi Motlagh et al. 2008; Syron and Casey 2008), pH and alkalinity (Shanahan & Semmens 2015), COD/N ratio (Carlson et al. 2021), and mixing intensity (Schraa et al. 2018). The mass transport of substrates, detachment processes of MAB biofilms, and attachment of suspended solids (Brannock et al. 2010; Qi et al. 2013; Plascencia-Jatomea et al. 2015) have also been modeled. Downing et al. (2017) investigated the impacts of adding an MABR in an unaerated zone in the Ejby Molle facility in Odense, Denmark. Their results revealed the feasibility of achieving shortcut nitrogen removal in the biofilm by manipulating the intramembrane air pressure. The process model also predicted more than a 50% reduction in energy and a 40% increase in peak flow capacity with the incorporation of MABRs. A recent study investigated the possibility of using MABRs to produce nitrate coupled with heterotrophic denitrification from suspended biomass in a largely anoxic suspended growth bioreactor (Carlson et al. 2021). This study provided a proof of concept in sizing the MABR to accomplish a substantial proportion of the ammonia removal via nitrification, which significantly reduced or even eliminated aerated zones while still achieving effective BNR performance.

The unique oxygen transfer scheme in MABRs can also be modeled explicitly. Houweling & Daigger (2019) described two methods: (1) the pressure-based model and (2) the exhaust oxygen-based model. (Peeters et al. 2017a, 2017b) used a version of the pressure-based model to account for variations in the oxygen partial pressure along the length of the MABR fiber.
Guglielmi et al. (2020) developed an exhaust oxygen-based model to monitor the key performance indicator OTR, which correlated with the ammonia removal rate in the biofilm. Even though both modeling methods have been used in commercial MABR applications, each is associated with biases. For the pressure-based model, the oxygen concentration at the base MAB significantly influences the oxygen transfer fluxes through the membrane into the biofilm, but the measurement of the oxygen concentration in the base layer can be difficult in practical applications (Houweling & Daigger 2019). On the other hand, current exhaust oxygen-based models ignore nitrogen gas diffusion across the membrane into the biofilm, and because of that, the predicted OTEs may be underestimated (Houweling & Daigger 2019). More detailed oxygen transfer models can undoubtedly be formulated and developed. Still, the benefits of the resulting more complex models need assessments relative to the needs for various modeling objectives.

To date, most MABR models are one-dimensional (1D), which has limitations to reflect the biological processes in MABs accurately. Non-uniform distribution of the DO concentration, microbial composition, and biofilm characteristics along the membrane fibers of the MABR poses a significant challenge for accurately modeling MABRs. An MABR model excluding the longitudinal heterogeneity of the biofilm may lead to biased evaluation of N2O production (Chen et al. 2020) and nitrogen removal (Acevedo & Lackner 2019). To account for the longitudinal heterogeneity of the MAB, dynamic and multi-dimensional models are needed. Schraa et al. (2018) developed a dynamic MABR model that predicted the performance of a pilot-scale MABR system for 220 days under dynamic operational conditions. The dynamic MABR model allowed non-uniform biofilm geometries so that a fixed thickness did not constrain the predicted biofilm thickness. In addition, a two-dimensional (2D) model was developed to simulate autotrophic denitrification in a spiral wound MBfR (Martin et al. 2013). The results highlighted the importance of using multi-dimensional models to capture the uneven biofilm thickness and density along the membrane. However, dynamic and multi-dimensional models increase modeling complexity, resulting in outstanding computational efforts (Wanner et al. 2006). Thus, it is essential to consider if a complex multi-dimensional model is necessary or whether a simplified model could answer the same question.

Extracellular polymeric substances (EPS) are fundamental constituents of the biofilm structure (Kreft & Wimpenny 2001). To date, few models have explicitly defined the role played by the EPS matrix in MABs (Boltz et al. 2017). Hu et al. (2020) reported that EPS production in a pure biofilm MABR system for phenol degradation was mediated by quorum-sensing systems. Production of EPS in Hybrid MABR/AS processes might be different from that in pure biofilm systems, but a systematic understanding is still lacking (SI). Further research needs to characterize EPS production in pure biofilm and hybrid MABR applications examining factors such as gene expression, impacting factors, and production rates.

Another knowledge gap is the need to implement dynamic detachment to predict the effects of air scouring in layered MABR models. Biofilm detachment typically is modeled by detaching the outermost biofilm layer, but in reality, not just the outer layer leaves from the biofilm (Petrova & Sauer 2016). A relevant question is the movement of microbial species within the biofilm, which affects their net retention in the process. Aerobes enriched in the inner layers are likely to move to outer layers, and in this case, air scouring can lead to detachment of nitrifiers. In addition, inner layers of the biofilm can also slough off due to structural weaknesses resulting from voids and cell dispersion (Petrova & Sauer 2016; Aybar et al. 2019; Kim et al. 2020). Considering these factors during model development is important to improve accuracy and reduce biases, but they also increase model complexity and runtimes. Research questions also remain concerning how to model microbial behaviors and how to validate such models.

5. PERFORMANCE ASSESSMENT

5.1. Biological carbon and nutrient removal

MABRs are a competitive alternative for process intensification in water resource recovery facilities (WRRFs) to treat greater substrate loads and achieve higher effluent qualities without additional footprint (Kunetz et al. 2016). Table 2 summarizes the reported NRs and carbon removal rates from the selected pilot- to full-scale studies. MABRs enable high NRs in the biofilm, which is beneficial for plants to achieve effective ammonia removal. A pilot plant in Ontario, Canada using the Zeelung Hybrid MABR/AS process reported an NR of 2.6 g N/m²-d in the biofilm (Peeters et al. 2017a, 2017b). Similarly, the full-scale spirally-wound hybrid MABR achieved an average NR of 3.1 g N/m²-d in the biofilm (Nathan et al. 2020). In fact, NRs in Hybrid MABR/AS systems were generally within the range of 1–3 g NH₄-N/m²-d (Table 2). In comparison, a typical NR in Hybrid MBBR/AS system is 0.5 g N/m²-d (Houweling & Daigger 2019).
A tradeoff also exists between TN and ammonia removal, and higher COD/N ratios in the influent favor TN removal but reduce NRs. Nitrification products in the hybrid MAB are exported to the bulk liquid where nitrate and nitrite are consumed together with the biodegradable organic matter by the suspended heterotrophic biomass (Downing et al. 2008). As discussed in section 2, the hybrid process represents an effective approach to reduce the competition between heterotrophs and

| Configuration | Wastewater type                  | Membrane type                             | Influent wastewater                      | MABR unit HRT (hrs) | NR (g N/m²d) | Carbon removal (%) | Suspended growth SRT (d) | Reference               |
|---------------|----------------------------------|-------------------------------------------|------------------------------------------|---------------------|--------------|---------------------|--------------------------|-------------------------|
| One-stage MABR | Municipal landfill leachate, Ireland | Dense polydimethylsiloxane (PDMS) membrane | sCOD: 1,000–3,000 mg/L, NH₄⁺-N: 500–2,500 mg/L | 108–156             | 1.0–1.59     | Effluent sCOD: 200–500 mg/L | NA                       | Syron et al. (2015)     |
| One-stage MABR | Municipal wastewater with industrial loads, Ontario, Canada | OxyMem MABR PDMS membrane | sCOD/NH₄⁺-N: 9.2–21.5 | 5.2–18             | 0.24–0.60     | 26–64% sCOD removal | NA                       | Bicudo et al. (2019)    |
| One-stage MABR | Municipal wastewater PE+ RAS, North America* | ZeeLung MABR dense membrane | NA | 0.38 | 2.2 | NA | NA | Houweling et al. (2017) |
| One-stage MABR | Municipal wastewater sidestream, Israel | Fluence MABR membrane | NH₄⁺-N: 250 mg/L | NA | 6–7 | NA | NA | Shechter et al. (2020a, 2020b) |
| Hybrid MABR in the anoxic zone in the A²O process | Municipal wastewater, Israel | Fluence MABR membrane | sCOD/NH₄⁺-N: 10.1 | NA | 1.3–5.5, average: 3.1 | NA | NA | Nathan et al. (2020) |
| Hybrid MABR in the anoxic zone in the A²O process | Municipal wastewater, Ontario, Canada | ZeeLung dense MABR membrane | sCOD/NH₄⁺-N: 0.96–5 | 7.5 | 1.2–2.6 | 77.5% sCOD removal | 4–8 | Peeters et al. (2017a, 2017b) |
| Hybrid MABR in the anoxic zone in the A²O process | Municipal wastewater, USA | ZeeLung MABR dense membrane | BOD₅/ NH₄⁺-N:5.6 | NA | Average: 1.6 Peak: 3 | 42% filtered BOD₅ removal | 10 | Kunetz et al. (2016) |
| Hybrid MABR in the anoxic zone in the A²O process | Municipal wastewater, USA | ZeeLung MABR dense membrane | BOD₅/ NH₄⁺-N:9.3 | 0.32 | 2.36 | Not reported | 10–12 | Underwood et al. (2018) |
| Hybrid MABR in the anoxic zone in the A²O process | Municipal wastewater, USA | ZeeLung MABR dense membrane | BOD₅/ NH₄⁺-N:8.4 | 0.32 | 2.1, up to 3.1 | Not reported | 12 | Guglielmi et al. (2020) |
| Hybrid MABR in the anoxic zone in the MLE process | Municipal wastewater, Sweden | ZeeLung MABR dense membrane | sCOD: 30–120 mg/L, NH₄⁺-N: 10–22 mg/L | 2.4–3.6 | 0.37–5.93 | 56.66% sCOD removed | 12–14 | Li (2018) |
| 3-stage Hybrid MABRs in the anoxic zone in the MLE process | Municipal wastewater, Canada | ZeeLung MABR dense membrane | sCOD/NH₄⁺-N: 0.23–1.35 | 7.5 | 1–3 | NA | 7.5 | Côté et al. (2015) |
nitrifiers, which can explain the consistent and stable nitrification performance observed in various hybrid systems (Downing & Nerenberg 2008a, 2008b). NRs in pure biofilm systems (e.g., one-stage MABRs) can vary significantly depending on influent wastewater characteristics. As ammonia and biological organic carbon are metabolized together in the biofilm, it is commonly observed that the NRs in pure biofilm systems are more susceptible to the influent COD/N. At high COD/N ratios, AOBs can be outcompeted by heterotrophic bacteria, while at low COD/N ratios, nitrification would proceed but the availability of electron donors can limit denitrification. Therefore, the relative concentrations of ammonia and biodegradable organic carbon are critical for SND in pure biofilm systems. A former study reported that a COD/N ratio of 4 was optimal for SND in an one-stage MABR (LaPara et al. 2006), but COD/N ratios and biodegradability of the given carbon source in different applications vary greatly. In this case, more elaborated biofilm and oxygen control strategies are needed to develop the desired stratification in pure biofilm systems.

The Hybrid MABR/AS process is a typical configuration to intensify biological carbon and nutrient removal in plants. Incorporating MABRs in the unaerated zones creates an aerobic condition in the biofilm for nitrification, and the produced nitrate is released into the anoxic bulk liquid where the suspended biomass performs denitrification for complete nitrogen removal (Houweling et al. 2017). The SND pathway has been well-demonstrated in pilot-scale testing (Kunetz et al. 2016; Peeters et al. 2017a, 2017b) and full-scale applications (Houweling et al. 2017; Underwood et al. 2018; Nathan et al. 2020). Nathan et al. (2020) reported that 97% of the ammonia removed in the MABR zone was denitrified in the same tank. In 2017, an Oxymem MABR was installed on-site to expand the capacity of its aerobic lagoons (Heffernan et al. 2017). The hybrid MABR system facilitated SND in a single bioreactor, removing 85% for total COD, 88% for ammonia, and 68% for TN (effluent quality: 44 mg/L total COD and 16 mg/L TN). The SND facilitated by hybrid MABRs increases treatment capacity within a given tank volume, which, combined with significant energy savings, makes the hybrid MABR a promising alternative for process intensification.

Hybrid MABR/AS processes can be operated at a lower suspended growth SRT than conventional AS systems, which translates into potentially lower footprints and reduced construction costs (Houweling & Daigger 2019; Carlson et al. 2021). A pilot Hybrid MABR/AS system at the Hayward Water Pollution Control Facility achieved 25%–30% TN removal at a low SRT of 1.5 days, typically too short for nitrogen removal in conventional AS systems (Sathyamoorthy et al. 2019). Researchers hypothesized that the ability to nitrify at a low SRT (usually below the washout SRT of nitrifiers) in the Hybrid MABR/AS system was due to the seeding effect from the MABR media, which supplemented nitrifying populations in the suspended biomass (Sunner et al. 2018). This theory has been validated in a comparative study where the downstream bioreactor, seeded by the MABR, fully nitrified at an SRT below the washout SRT of nitrifiers (Houweling et al. 2018). Process modeling in the same study further identified that the fraction of ammonia removed in the biofilm and the sloughed yield of nitrifiers from the biofilm were the most significant influencing factors for the seeding effect. Similarly, Houweling et al. (2017) demonstrated the robustness of the hybrid process at an SRT less than five days under diurnal loading variations: with a peak loading factor of 1.9 and an influent ammonia concentration of 5–50 mg/L, the effluent ammonia was less than 1 mg/L 45% of the time and less than 2 mg/L 94% of the time.

Biological nitrogen removal processes can be a significant source of the potent greenhouse gas N₂O (Conthe 2019; Duan et al. 2021). In MABRs where ammonia and oxygen diffuse into the biofilms from opposite directions, N₂O formation mainly occurs in the inner biofilm portion by AOBs via the hydroxylamine oxidation pathway (Kinh et al. 2017a, 2017b; Sabba 2018). In addition, heterotrophic denitrifiers via the incomplete denitrification pathway may also contribute N₂O production in the transition zone from aerobic to anoxic within the MAB (Pellicer-Nächter et al. 2010). This occurs because the NOS enzyme that reduces N₂O to N₂ can be selectively inhibited by oxygen (Morley et al. 2008). However, compared to co-diffusional biofilm reactors, significantly less N₂O emissions and accumulation in the biofilm-liquid interface have been observed in MABRs, due to the adjacent positions of the N₂O formation and degradation zones (Kinh et al. 2017a, 2017b). A former study indicated that heterotrophic bacteria, e.g., Thauera and Rhizobium, could reduce the N₂O produced in the inner biofilm layers (Kinh et al. 2017a, 2017b).

To date, several conditions are known to promote N₂O formation in the biological nitrogen removal process: (1) low or fluctuating DO concentrations, which leads to nitrite reduction by AOBs, (2) high nitrogen loadings that lead to the greater formation of intermediates (e.g., hydroxylamine and nitrite); or (3) limited carbon sources which lead to incomplete denitrification (Sabba 2018). Even though MABRs can mitigate N₂O emissions compared to conventional biofilm reactors, some N₂O produced in the MAB can still be stripped into the gas lumen and release into the air if operated in the flow-through
aeration mode (Kinh et al. 2017a, 2017b). A question is whether the exhaust gas stream can be practically captured and the N$_2$O contained in it can be controlled.

EBPR occurs in Hybrid MABR/AS systems where an additional anaerobic zone is added prior to the anoxic MABR zones (Sathyamoorthy et al. 2019). A benefit of performing EBPR in a Hybrid MABR/AS system is that the SND in the MABR zone can decrease nitrate return to the anaerobic zone and reduce its interference with PAO activities. Robust EBPR activity was observed in a full-scale Hybrid MABR/AS process in Yorkville, IL with an average effluent total phosphorus (TP) concentration of 0.49 mg/L (Underwood et al. 2018). The same study reported that the SND in the MABR decreased the nitrate return to the anaerobic zone and reduced its interference with EBPR. Similarly, the pilot Hybrid MABR/AS in the O’Brien Water Reclamation Plant showed that a TP limit of 1 mg-P/L could be sustained with existing plant infrastructure (Kunetz et al. 2016). In addition, a pilot plant study at the Ekeby WWTP (Eskilstuna, Sweden) recorded a >65% removal of TP without the use of coagulants or a well-defined anaerobic zone, despite phosphorus removal being an auxiliary design objective (Li 2018). Overall, phosphorus removal has been observed in MABRs, but it is still an underdeveloped topic in MABR systems. More research needs to investigate the conditions under which optimal EBPR can occur and the effects of MABRs on both PAO and DPAO activities.

5.2. Autotrophic nitrogen removal

Autotrophic nitrogen removal via amamnox microorganisms is an emerging application of MABRs. Lab-scale studies using synthetic feed have demonstrated the feasibility to achieve PN/A via an one-stage MABR where AOB are abundant in oxygen-rich layers close to the lumen while anammox bacteria are located in the anoxic zone closer to the bulk liquid (Gong et al. 2007; Pellicer-Nàcher et al. 2014; Augusto et al. 2018). In a recent lab-scale study, an one-stage MABR was used to treat municipal wastewater under mainstream conditions (Bunse et al. 2020). Under low ammonia and COD concentrations (31–120 mg N/L, 7–250 mg sCOD/L), the MABR achieved an average ammonia removal rate of 2.3–3.6 g N/m$^2$.d and a TN removal rate of 1.2 g N/m$^2$.d. Excellent nitrogen removal by MABRs performing PN/A was also observed in a recent pilot treating digestate from the side stream of a WRRF (Coutts et al. 2020). This pilot achieved an ammonia removal rate of 5.5 g N/m$^2$.d and a TN removal rate of 4.4 g N/m$^2$.d. Overall, MABRs performing PN/A show good performance and considerable energy savings (energy consumption of 0.4 kWh/kgN (Coutts et al. 2020). However, excessive growth of NOBs and nitrate build-up are challenges for PN/A (Lackner et al. 2014). Online monitoring and control will be critical to ensuring process resilience and optimization of MABRs performing PN/A.

Previous studies confirmed the coexistence of anammox bacteria, AOB, NOB, and heterotrophic bacteria in PN/A MABRs (Gong et al. 2007; Ma et al. 2017a, 2017b; Zhao et al. 2021). Because of the complex microbial community and competing reactions, the manipulation of operational parameters is critical to creating an environment for the anammox bacteria’s optimal growth. For instance, anammox bacteria are sensitive to DO levels, and the presence of oxygen also promotes NOB growth and competition for nitrite, hampering PN/A performance (Strous et al. 1997; Kuenen 2008). Too little oxygen, on the other hand, limits AOB growth, leading to insufficient nitrite production (Cema et al. 2011). Therefore, oxygen mass transfer is a key factor that determines the success of PN/A.

In MABRs, transmembrane gas pressure is often manipulated to control oxygen transfer (Gong et al. 2007; Zhao et al. 2021). Wang et al. (2016) reported that an increase in transmembrane gas pressure from 2 to 5 kPa increased nitrite accumulation from 2.8 to 7.4 mg/L, indicating successful suppression of NOBs. However, a further increase to 20 kPa resulted in excessive oxygen supply and unwanted full nitrification. Nevertheless, control of the transmembrane gas pressure may not be suitable to sustain nitrification in long-term operation because of the accumulation of K-strategist NOBs, such as Nitrospira, under continuous low DO conditions (Gilmore et al. 2013; Gilbert et al. 2014). Therefore, intermittent aeration is commonly used in MABRs as a long-term solution to suppress NOBs (Pellicer-Nàcher et al. 2014; Ma et al. 2017a, 2017b). Pellicer-Nàcher et al. (2014) observed a considerable decrease in NOB-related 16S rRNA gene abundance and a considerable increase in anammox 16S rRNA gene abundance, when intermittent aeration was implemented in a one-stage MABR. Additionally, the ZeeNAMMOX process reversed the vertical oxygen supply direction in the MABR lumen periodically to switch the DO limited condition from top to bottom, suppressing NOB growth by periodic exposure to anoxic conditions (Long et al. 2020). As was indicated by the ratio of nitrate generation to ammonia oxidation of 0.2, which is relatively close to the stoichiometric ratio for PN/A (0.11) (Strous et al. 1998), the pilot ZeeNAMMOX process achieved excellent NOB suppression, and the specific total inorganic nitrogen (TIN) removal rates reached 6–10 g N/m$^2$.d. Overall, management of oxygen in the MABR lumen is critical to enrich AOBs and anammox while selectively suppressing NOBs.
Achieving stable nitrification by manipulating the oxygen availability in long-term operation is still challenging, and further research needs to optimize the aeration intermittency and duration.

Ammonia loading is another critical factor that affects PN/A in MABRs. Although free ammonia (FA) and nitrite are essential substrates for anammox processes, high concentrations of FA and nitrite can be toxic and inhibitory to anammox activity (Cho et al. 2019). Lackner et al. (2014) reported that a higher availability of nitrite (higher than 0.07 g N/L) at the startup stage can interrupt anammox growth. Increased ammonia loadings have also been shown to complicate process operation and control, as the oxygen flux is typically increased proportionally to the increase in ammonia loading to ensure sufficient ammonia oxidation, which may pose problems to the microbial community. For example, in a one-stage MABR system fed with synthetic wastewater, increasing the ammonia loading rate from 50 to 100 g N/m²d under the same DO condition resulted in a drop in the TN removal efficiency from 84% to 69% (Augusto et al. 2018). Decreased removal performance was attributed to the increased airflow with DO spikes under the increased nitrogen loading, which might have allowed NOBs to proliferate. Although it may be advantageous to perform PN/A in counter-diffusional MABs because of the independent control of oxygen and ammonia availability, advanced monitoring and control strategies must be applied to keep the balance.

Low DO concentrations favored by the PN/A process are also conducive to the unwanted formation of N₂O (Ma et al. 2017a, 2017b). Compared to SND MABRs, more N₂O can be potentially produced in PN/A MABRs where DO limiting conditions are intentionally created to suppress NOB growth. However, as discussed in section 5.1, MABRs emit and accumulate less N₂O at the biofilm-liquid interface, which is likely due to the adjacent positions of the N₂O formation and degradation zones (Wang et al. 2016; Kinh et al. 2017a, 2017b). Similar to biological nitrogen removal via SND, N₂O in PN/A MABRs may derive predominantly from AOBs in the inner MAB (Pellicer-Ñácher et al. 2010; Gilmore et al. 2013). Besides hydroxylamine oxidation via AOBS, N₂O may also be produced by nitrite reduction during denitrification (Khalil et al. 2004). Overall, avoiding simultaneous low DO and high nitrogen loadings in PN/A MABRs is vital for minimizing N₂O formation (Sabba 2018). In this case, one-stage MABRs with continuous nitrite consumption under low DO conditions should have a lower N₂O footprint than two-stage MABRs, which requires a significant nitrite residual conveyed to the separate anammox reactor. However, N₂O formation is a complex process that involves multiple microbial groups, and factors that influence the N₂O formation in PN/A MABRs are not always intuitive. Future investigations need to characterize the production and mitigation mechanisms in MABRs performing the PN/A process.

5.3. Space-based wastewater treatment

Federal programs have assessed MABR technology into low or no-gravity wastewater treatment applications over the last two decades at both pilot- and full-scale (Morse et al. 2004; Chen et al. 2008; Christenson et al. 2018). It is envisioned that MABRs could provide unique benefits to the closed-loop recycling of wastewater to potable water in missions outside of Earth, including the upcoming Artemis program destined for Earth’s moon. At a fundamental level, the mechanism of oxygen delivery used by MABRs avoids one of the significant pitfalls of conventional aeration in an environment without gravity: bubbles. Since oxygen transfer from the membrane is driven by a concentration gradient, microbial growth can occur in both micro-gravity (e.g., Lunar or Martian) or reduced gravity conditions (Jackson et al. 2009; Landes et al. 2021).

MABR design and operation can enhance health and safety, logistics, and functionality in non-terrestrial systems. The integration of MABRs would minimize the use of toxic chemicals, which are typically used to pretreat the high concentrations of urea and ammonia in space-based wastewater. Historical pilot studies conducted by Chen et al. (2008) and Jackson et al. (2009), using a carbon limited and nitrogen dominant wastewater at small loading rates that reflect the characteristics of a space mission, suggested promising SND with ammonia and COD removal efficiencies up to 90%. When a full-scale MABR (the Counter-diffusion Membrane Aerated Nitrifying and Denitrifying Reactor or CoMANDR) was designed for integration with critical water recovery systems, the unit achieved 90% carbon oxidation and 60% nitrification efficiencies at a hydraulic residence time of approximately 5 days (Christenson et al. 2018). Furthermore, the system was tested during a separate ‘hibernation’ phase and rapid startup to regular operation, where necessary systems were shut off for nearly a month and then returned to normal system feed, which are typical during space missions. Christenson et al. (2018) found that the CoMANDR system could recover quickly after hibernating for almost a month and was well-suited as an upstream treatment step in a water recovery scheme. In a most recent lab-scale study, the MABR was designed to treat synthetic space-based wastewater with limited total organic carbon (TOC) and concentrated ammonia (TOC/N>1) (Landes et al. 2021). Successful SND was observed in this study with a TN removal efficiency of 36.5% and removal rate of 0.24 g N/m²d.
The uniqueness of this application of MABR technology creates an opportunity for continuing research. The small number of crew on space missions limits the hydraulic loading on the system, allowing for batch systems to be a possibility. However, in contrast to municipal wastewater, the low COD/N ratio (<2) in space-based wastewater hampers denitrification, as stoichiometric limitations are shown to be major obstacles in MABR application (Landes et al. 2021). Finally, the effluent produced from an MABR used in a non-terrestrial application must be of the highest quality and readily compatible with downstream treatment processes for potable water generation.

5.4. High-strength industrial wastewater and xenobiotics biotreatment

MABRs are advantageous to treat high-strength, industrial wastewater due to their high OTEs and OTRs, tolerance of high salinity, ability to degrade intermediates in multiple redox gradient zones, and minimized stripping of volatile organic compounds (VOCs) (Quan et al. 2018; Hu et al. 2020; Tian et al. 2020). A lab-scale MABR system treating synthetic high-strength swine wastewater (4500 mg COD/L, 4000 mg TN/L) achieved 96% COD removal and 83% TN removal at removal rates of 5.8 g COD/m²-d and 4.5 g N/m²-d (Terada et al. 2003). A similar COD removal rate (6 g COD/m²-d) was reported in a pilot-scale MABR system treating a synthetic high-strength industrial wastewater (4700 mg COD/L, 145 mg TKN/L). Even though a low NR of 0.04–0.09 g N/m²-d was reported in this pilot due to the high COD/N ratio, 76%–85% of the nitrite and nitrate produced were immediately denitrified, with an overall denitrification efficiency of 94%.

MABRs are beneficial to treat xenobiotics because that MABs can sustain substrate degradation rates from industrial wastewater loads, as the microbes embedded in the biofilm are protected by the outer EPS matrix (Abdelfattah et al. 2020). Specifically, McLamore et al. (2010) analyzed the cellular stress response of a counter-diffusional MAB under increasing loadings of toxins. The study found that exposure to higher concentrations of toxins led to increased oxygen and proton flux into the biofilm, which might be a defense mechanism for survival.

A number of laboratory studies have investigated the degradation of xenobiotics with MABRs, including fluorinated organics (Heffernan et al. 2009; Misiak et al. 2011), phenolic compounds (Mei et al. 2019; Tian et al. 2019; Tian et al. 2020), dyes (Wang et al. 2012), and organonitrile compounds (Li & Liu 2019). Figure 4 illustrated the treatment performance of those MABR studies. Tian et al. (2019) used a two-stage MABR system for o-aminophenol and nitrogen removal. The effective removal of o-aminophenol in the first MABR (removal rate of 17.6 g/m²-d) mitigated the inhibitory effect of xenobiotics on the nitrifiers, and therefore good nitrogen removal efficiency of 90% was achieved in the second MABR. A single MABR used to treat multiple phenolic compounds achieved a removal rate of 8.9 g/m²-d for total phenolics (Tian et al. 2020). In a separate study, an acetonitrile (ACN) removal efficiency of 98% was achieved in an one-stage MABR with an hydraulic residence time (HRT) of 6 h, which corresponded to an ACN removal rate of 3.63 g/m²-d (Kunlasubpreedee & Visvanathan 2020). Besides the application for single contaminant removal, another research team reported the capability

![Figure 4](http://iwaponline.com/wst/article-pdf/doi/10.2166/wst.2021.443/943189/wst2021443.pdf)
of an MABR system to treat a complex pharmaceutical wastewater mixture with 90% COD removal and 98% ammonia removal (Wei et al. 2012). Overall, both one- and two-stage MABR applications achieved good removal efficiencies of industrial pollutants. Their demonstrated resilient performance opens the possibility of applying MABRs to treat more emerging pollutants, and such potential applications need further investigation.

A long SRT operation is usually necessary to develop sufficient biomass for industrial compounds removal because of their low biodegradability and inhibitory effects. As a result of the long SRT, a thicker biofilm is likely to develop (SI). In addition, the high-strength industrial wastewater is associated with high organic carbon loading rates, and thereby supplying pure oxygen into MABRs may be beneficial to improve COD removal efficiency. As was discussed in section 2.3, thicker biofilms also grow under high loadings and pure oxygen feed. All these factors may lead to an excessively biofilm accumulation that is prone to mass transfer resistance issues that decrease the activity of the biofilm. Stricker et al. (2011) reported that intermittent air sparging failed to control the biofilm thickness in an MABR treating high-strength industrial wastewater, leading to performance upset. An explanation was that, after air scouring events, the promoted mass transfer in the thinner biofilm resulted in faster diffusion of the highly concentrated substrates and quick accumulation of biomass. Therefore, more aggressive and continuous air scouring may be needed for biofilm thickness control in MABRs for high-strength industrial wastewater treatment. Still, investigations are required to understand biofilm responses to such events.

5.5. Sulfur recovery
MABR technology represents an efficient aerobic method to deliver oxygen directly into the biofilm formed by sulfide-oxidizing bacteria (SOB) (Sun et al. 2017). The complete oxidation of sulfide to sulfate is a favored metabolism for SOB as the process yields more energy compared to the partial oxidation of sulfide to elemental sulfur, and thereby the successful operation of biological elemental sulfur recovery relies on a delicate balance between oxygen and sulfide availability to SOB to stop biological sulfide oxidation at elemental sulfur (Cai et al. 2017). The counter-diffusional geometry in MABRs is an efficient means of controlling the oxygen supply independent of sulfide availability which creates an oxygen-limited condition that favors elemental sulfur production. A good sulfur recovery rate (>75%) in MABRs has been successfully demonstrated in both experimental studies and mathematical modeling by controlling the combination of membrane oxygen pressure, HRT, and sulfide loading rate (Sahinkaya et al. 2011; Sun et al. 2017).

A previous study analyzed the competition between aerobic heterotrophs and SOB for oxygen in MABRs, and the results suggested that SOB were better scavengers for low DO concentrations than heterotrophs (Sahinkaya et al. 2011). This finding was consistent with a multispecies modeling study, where SOBs outcompeted heterotrophs under low oxygen flux conditions (Jiang et al. 2019). Camiloti et al. 2019 reported that Geovibrio, Flexispira, and Sulfurospirillum were key functional sulfide oxidation genera in the MAB.

To summarize, MABRs are promising for sulfur recovery from wastewaters. Nevertheless, this research topic is still at its early stage, and more studies need to analyze different impacting factors, e.g. presence of VFAs and nitrate, and different aeration strategies. In addition, as the produced elemental sulfur is colloidal and hydrophilic (Cai et al. 2017), solutions are needed to efficiently separate and recover the sulfur from MABs.

6. CURRENT CHALLENGES AND OUTLOOK
Investigations of the first generation of commercial MABR products show that MABR processes are capable of efficient treatment of a range of pollutants (COD, N, P, xenobiotics), advantageous for resource recovery (e.g., sulfur), capable of mitigating N₂O emissions, and beneficial for carbon and energy savings. The compact size of the MABR unit makes it easy to be installed in existing facilities and intensifies treatment performance without additional footprints. The demonstrated benefits provide incentives to enhance the performance capabilities further and lower the cost of subsequent generations of commercial products. Researchers have also investigated novel combinations of MABRs with other technologies, including microbial electrolysis cells (Pape et al. 2020) and membrane bioreactors (MBRs) (Daigger 2020). Novel bacterial-algae biofilms have also been developed in MABRs to treat wastewater of wider COD/N ratios (Zhang et al. 2021). It is foreseeable that the expanded use of MABRs and novel treatment processes will result in new research questions to be pursued, such as adequate mass transfer models, dynamic biofilm attachment/detachment, resilience design, and life cycle analysis (LCA).

Several existing challenges need ongoing research to improve future applications of MABRs. Firstly, solutions are needed to increase packing density while retaining efficient external mass transfer characteristics and avoiding solids build-up. This will lead to more compact units and further reductions in system size and cost. Secondly, a systematic understanding of functional
pathways, particle attachment/detachment mechanisms, and correlation between ecology niches and operating conditions is likely to improve biofilm control and treatment performance. In addition, microbial interactions between the attached and suspended growth, including cross-feeding, competition, and biomass exchange, occur dynamically under different conditions. A holistic view of the whole community requires comparative analyses across multiple information levels (biological, chemical, physical, and mechanical) to diagnose system states and predict system performance. Thirdly, process optimization is vital to fulfilling the design objectives but remains a challenge. As MABRs have the potential to achieve simultaneous COD and nutrient removal, fundamental understanding of their mechanisms needs to be improved for operations and configuration design compared to the conventional AS processes. Site-specific issues and treatment tradeoffs must be also considered to optimize the operational conditions.

Process control is critical to assist in the automation of MABR operation and decision-making. For this to occur, MABR models must be improved to account for dynamic spatial and physiological heterogeneity in the MAB of reasonable computational intensity. Moreover, it is challenging to decouple the interactions between biofilm and suspended biomass; therefore, the biofilm and suspended biomass in MABRs cannot be controlled independently. More advanced control approaches, such as model predictive control (MPC) (Zeng & Liu 2015) and data-driven control (Newhart et al. 2019), may be applied in future MABR studies to address the issues brought by complex interactions between biofilm and suspended biomass. In addition, artificial intelligence (AI) algorithms represent a robust alternative to control and optimize wastewater treatment processes (Zhao et al. 2020). New research in the utilization of AI in MABRs may increase the understanding of MABR operation, which could be used to improve process control.

7. CONCLUSIONS
MABR technology, whether operating in a pure biofilm or a hybrid process, is a promising technology for wastewater treatment. The bubble-less aeration enables higher oxygen transfer rates and efficiencies, leading to significantly reduced aeration costs. Due to the unique oxygen mass transfer scheme and microbial population stratification developed in the MAB, biomass inventory and removal fluxes are promoted within the compact MABR cassettes, intensifying the treatment capacity of existing facilities in a given reactor volume.

Studies in microbial community ecology and process models for MABRs have improved our understanding of the counter-diffusional biological process. Increased applications of MABR at pilot- and full-scales have proven that this technology is beneficial for removing a range of pollutants (COD, N, P, xenobiotics) and advantageous for resource recovery (e.g., sulfur). The unique microbial stratification can also mitigate N2O emissions, which is an emerging issue of concern for the BNR process. As MABR technology is rapidly evolving for wastewater treatment, conclusions at this stage are preliminary. Further research is needed to characterize microbial interactions between the biofilm and suspended growth in hybrid systems, address existing assumptions for improved MABR biofilm modeling and process control, and optimize the operational conditions that govern MABR performance.

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DATA AVAILABILITY STATEMENT
All relevant data are included in the paper or its Supplementary Information.

REFERENCES
Abdel fattah, A., Hossain, M. I. & Cheng, L. 2020 High-strength wastewater treatment using microbial biofilm reactor: a critical review. World Journal of Microbiology and Biotechnology 36 (5), 75.
Acevedo Alonso, V. & Lackner, S. 2019 Membrane aerated biofilm reactors – how longitudinal gradients influence nitrogen removal – A conceptual study. Water Research 166, 115060.
Ahmadi Motlagh, A. R., LaPara, T. M. & Semmens, M. J. 2008 Ammonium removal in advective-flow membrane-aerated biofilm reactors (AF-MABRs). Journal of Membrane Science 319 (1–2), 76–81.
| Authors | Title | Journal Details |
|---------|--------|-----------------|
| Akiyama, M., Crooke, E. & Kornberg, A. | An exopolyphosphatase of Escherichia coli. The enzyme and its ppx gene in a polyphosphate operon. | Journal of Biological Chemistry 268 (1), 633–639. |
| Arun, V., Mino™, T. & Matsuo™, T. | Biological mechanism of acetate uptake mediated by carbohydrate consumption in excess phosphorus removal systems. | Water Research 22 (5), 565–570. |
| Augusto, M. R., Camiloti, P. R. & Souza, T. S. O. d. | Fast start-up of the single-stage nitrogen removal using anammox and partial nitritation (SNAP) from conventional activated sludge in a membrane-aerated biofilm reactor. | Bioresource Technology 266, 151–157. |
| Aybar, M., Perez-Calleja, P., Li, M., Pavissich, J. P. & Nerenberg, R. | Predation creates unique void layer in membrane-aerated biofilms. | Water Research 149, 232–242. |
| Aybar, M., Pizarro, G., Martin, K., Boltz, J., Downing, L. & Nerenberg, R. | The air-based membrane biofilm reactor (MBfR) for energy efficient wastewater treatment. | Proceedings of the Water Environment Federation 2012 (10), 5458–5485. |
| Baskaran, V., Patil, P. K., Antony, M. L., Avunjie, S., Ghathe, S. D., Nathamuni, S., Dineshkumar, N., Alavandi, S. V. & Vijayan, K. K. | Microbial community profiling of ammonia and nitrite oxidizing bacterial enrichments from brackishwater ecosystems for mitigating nitrogen species. | Scientific Reports 10 (1), 5201. |
| Bolz, J. P., Smets, B. F., Rittmann, B. E., Van Loosdrecht, M. C. M., Morgenroth, E. & Daigger, G. T. | From biofilm ecology to reactors: a focused review. | Water Science and Technology 75 (8), 1753–1760. |
| Brannock, M., Wang, Y. & Leslie, G. | Mixing characterisation of full-scale membrane bioreactors: CFD modelling with experimental validation. | Water Research 44 (10), 3181–3191. |
| Brindle, K., Stephenson, T. & Semmens, M. J. | 1998 Nitrification and oxygen utilisation in a membrane aerated biofilm reactor. | Journal of Membrane Science. |
| Bunse, P., Orscherl, L., Agrawal, S. & Lackner, S. | Membrane aerated biofilm reactors for mainstream partial nitritation/anammox: experiences using real municipal wastewater. | Water Research X 9, 100066. |
| Cai, J., Zheng, P., Qaisar, M. & Zhang, J. | Elemental sulfur recovery from biological sulfide removal process from wastewater: a review. | Critical Reviews in Environmental Science and Technology 47 (21), 2079–2099. |
| Camiloti, P. R., Valdés, F., Delforno, T. P., Zaiat, M. & Jeison, D. | A membrane aerated biofilm reactor for sulfide control from anaerobically treated wastewater. | 3350. |
| Carlson, A. L., He, H., Yang, C. & Daigger, G. T. | 2021 Comparison of hybrid membrane aerated biofilm reactor (MABR)/suspended growth and conventional biological nutrient removal processes. | Water Science and Technology. wst2021062. |
| Carvalho, G., Lemos, P. C., Oehmen, A. & Reis, M. A. M. | Denitrifying phosphorus removal: linking the process performance with the microbial community structure. | Water Research 41 (19), 4383–4396. |
| Casey, E., Glennon, B. & Hamer, G. | 1999 Review of membrane aerated biofilm reactors. | Resources, Conservation and Recycling 27 (1–2), 203–215. |
| Casey, E., Syron, E. & Hefferman, B. | 2014 Determining Biofilm Thickness in A Membrane Supported Biofilm Reactor. | Water Research 152, 1–11. |
| Castrillo, M., Diez-Montero, R., Esteban-García, A. L. & Tejero, I. | 2019 Mass transfer enhancement and improved nitrification in MABR through specific membrane configuration. | Water Research 152, 1–11. |
| Cema, G., Plaza, E., Trela, J. & Surracce-Góriska, J. | 2011 Dissolved oxygen as a factor influencing nitrogen removal rates in a one-stage system with partial nitritation and Anammox process. | Water Science and Technology 64 (5), 1009–1015. |
| Chen, R. D., Semmens, M. J. & LaPara, T. M. | 2008 Biological treatment of a synthetic space mission wastewater using a membrane-aerated, membrane-coupled biofilm reactor (M2BR). | Journal of Industrial Microbiology & Biotechnology 35 (6), 465–473. |
| Chen, X., Yang, L., Sun, J., Wei, W., Liu, Y. & Ni, B. J. | 2020 Influences of longitudinal heterogeneity on nitrous oxide production from membrane-aerated biofilm reactor: a modeling perspective. | Environmental Science & Technology 54 (17), 10964–10973. |
| Cho, S., Kambe, C. & Nguyen, V. | 2019 Performance of anammox processes for wastewater treatment: a critical review on effects of operational conditions and environmental stresses. | Water 12 (1), 20. |
| Christenson, D., Sevanti, R., Morse, A. & Jackson, A. | 2018 Assessment of membrane-aerated biological reactors (MABRs) for integration into space-based water recycling system architectures. | Gravitational and Space Research 6 (2), 12–27. |
| Cole, A. C., Semmens, M. J. & LaPara, T. M. | 2004 Stratification of activity and bacterial community structure in biofilms grown on membranes transferring oxygen. | Applied and Environmental Microbiology 70 (4), 1982–1989. |
| Conthe, M. | 2019 Denitrification as an N2O sink. | Water Research 7. |
| Coutts, D., Di Poi, M., Baumgarten, S., Guglielmi, G., Peeters, J. & Houweling, D. | 2020 Side-Stream Treatment with Membrane Aerated Biofilm Reactors – the Simple, Robust and Energy Efficient Path. | Helsinki, Finland. |
| Daigger, G. | 2020 MABR workshop: current status and emerging applications. | In: IWA Biofilms 2020 Virtual Conference. |
| Daims, H., Lebedeva, E. V., Pjavec, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R. H., von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P. H. & Wagner, M. | 2015 Complete nitrification by Nitrospira bacteria. | Nature 528 (7583), 504–509. |
Delgado Vela, J., Bristow, L. A., Marchant, H. K., Love, N. G. & Dick, G. J. 2020 Sulfide alters microbial functional potential in a methane and nitrogen cycling biofilm reactor. *Environmental Microbiology*. 1462-2920.15352.

Dick, G. 2018 *Genomic Approaches in Earth and Environmental Sciences*. Wiley Blackwell.

Díez-Montero, R., De Florio, L., González-Viar, M., Herrero, M. & Tejero, I. 2016 Performance evaluation of a novel anaerobic–anoxic sludge blanket reactor for biological nutrient removal treating municipal wastewater. *Bioresource Technology* 209, 195–204.

Downing, L. 2021 *Next Generation Biofilms*. The Water Research Foundation. Available from: https://www.werf.org/resource/emerging-technologies-nutrient-optimization (accessed 27 May 2021).

Downing, L. S. & Nerenberg, R. 2008a Effect of oxygen gradients on the activity and microbial community structure of a nitrifying, membrane-aerated biofilm. *Biotechnology and Bioengineering* 101 (6), 1193–1204.

Downing & Nerenberg, R. 2008b Effect of bulk liquid BOD concentration on activity and microbial community structure of a nitrifying, membrane-aerated biofilm. *Applied Microbiology and Biotechnology* 81 (1), 153–162.

Downing, Bibby, K. J., Fascianella, T., Esposito, K. & Nerenberg, R. 2008 The hybrid membrane biofilm process for TN removal from wastewater: Bench and pilot scale studies. In: *World Environmental and Water Resources Congress 2008: Akupua’a – Proceedings of the World Environmental and Water Resources Congress 2008*. Vol. 316, pp. 1–10.

Downing, L. S., Bibby, K. J., Esposito, K., Fascianella, T., Tsuchihashi, R. & Nerenberg, R. 2010 Nitrogen removal from wastewater using a hybrid membrane-biofilm process: pilot-scale studies. *Water Environment Research* 82 (3), 195–201.

Downing, L. S., Willoughby, A., Constantine, T., Sandino, J., Uri, N. & Nielsen, P. 2017 Applying a disruptive technology: practical considerations for the MABR at the Ejby Molle facility in Odense, Denmark. *Proceedings of the Water Environment Federation* 2017 (16), 266–271.

Duan, H., Zhao, Y., Koch, K., Wells, G. F., Zheng, M., Yuan, Z. & Ye, L. 2021 Insights into nitrous oxide mitigation strategies in wastewater treatment and challenges for wider implementation. *Environmental Science & Technology* 55 (11), 7208–7224.

Flowers, J. J., He, S., Yilmaz, S., Noguera, D. R. & McMahon, K. D. 2009 Denitrification capabilities of two biological phosphorus removal sludges dominated by different ‘Candidatus Accumulibacter’ clades. *Environmental Microbiology Reports* 1 (6), 583–588.

Gao, H., Liu, M., Griffin, J. S., Xu, L., Xiang, D., Scherson, Y. D., Liu, W.-T. & Wells, G. F. 2017 Complete nutrient removal coupled to nitrous oxide production as a bioenergy source by denitrifying polyphosphate-accumulating organisms. *Environmental Science & Technology* 51 (8), 4531–4540.

Gilbert, E. M., Agrawal, S., Brunner, F., Schwartz, T., Horn, H. & Lackner, S. 2014 Response of different *Nitrosira* species to anoxic periods depends on operational DO. *Environmental Science & Technology* 48 (5), 2934–2941.

Gilmore, K. R., Little, J. C., Smets, B. F. & Love, N. G. 2009 Oxygen transfer model for a flow-through hollow-fiber membrane biofilm reactor. *Journal of Environmental Engineering* 135 (9), 806–814.

Gilmore, K. R., Terada, A., Smets, B. F., Love, N. G. & Garland, J. L. 2013 Autotrophic nitrogen removal in a membrane-aerated biofilm reactor under continuous aeration: a demonstration. *Environmental Engineering Science* 30 (1), 38–45.

Gong, Z., Yang, F., Liu, S., Bao, H., Hu, S. & Furukawa, K. 2007 Feasibility of a membrane-aerated biofilm reactor to achieve single-stage autotrophic nitrogen removal based on Anammox. *Chemosphere* 69 (5), 776–784.

Guglielmi, G., Coutts, D., Houweling, D. & Peeters, J. 2020 Full-scale application of mabr technology for upgrading and retrofitting an existing wwtp: performances and process modelling. *Environmental Engineering and Management Journal* 19 (10), 1781–1789.

Heffernan, B., Murphy, C. D., Syron, E. & Casey, E. 2009 Treatment of fluoroacetate by a *Pseudomonas fluorescens* biofilm grown in membrane aerated biofilm reactor. *Environmental Science & Technology* 43 (17), 6776–6785.

Heffernan, B., Shrivastava, A., Toniolo, D., Semmens, M. & Syron, E. 2017 Operation of a large scale membrane aerated biofilm reactor for the treatment of municipal wastewater. *Proceedings of the Water Environment Federation* 2017 (16), 285–297.

Hou, F., Li, B., Xing, M., Wang, Q., Hu, L. & Wang, S. 2013 Surface modification of PVDF hollow fiber membrane and its application in membrane aerated biofilm reactor (MABR). *Bioresource Technology* 140, 1–9.

Hou, D., Jassby, D., Nerenberg, R. & Ren, Z. J. 2019 Hydrophobic Gas transfer membranes for wastewater treatment and resource recovery. *Environmental Science & Technology* 53 (20), 11618–11635.

Houweling, D. & Daiggar, G. T. 2019 Intensifying Activated Sludge Using Media-Supported Biofilms. CRC Press. Available from: https://www.taylorfrancis.com/books/9780429522420 (accessed 2 March 2021).

Houweling, D., Peeters, J., Cote, P., Long, Z. & Adams, N. 2017 Proving membrane aerated biofilm reactor (mabr) performance and reliability: Results from four pilots and a full-scale plant. In: *Water Environment Federation Technical Exhibition and Conference 2017, WEFTEC 2017*. Vol. 5, pp. 3420–3432.

Houweling, D., Long, Z., Peeters, J., Adams, N., Côte, P., Daiggar, G. & Snowling, S. 2018 Nitrifying below the ‘Washout’ SRT: experimental and modelling results for a hybrid MABR/activated sludge process. *Proceedings of the Water Environment Federation* 2018 (16), 1250–1263.

Hu, Y., Hu, Y., Li, Y., Hui, M., Lu, Z., Li, H. & Tian, H. 2020 Metagenomic insights into quorum sensing in membrane-aerated biofilm reactors for phenolic wastewater treatment. *Environmental Technology, 1–10.*

Jackson, W. A., Morse, A., McLamore, E., Wiesner, T. & Xia, S. 2009 Nitrification-denitrification biological treatment of a high-nitrogen waste stream for water-reuse applications. *Water Environment Research* 81 (4), 423–431.

Janczewski, L. & Trusek-Holowmia, A. 2016 Biofilm-based membrane reactors – selected aspects of the application and microbial layer control. *Desalination and Water Treatment* 57 (48–49), 22909–22916.
Jiang, X., Xu, B. & Wu, J. 2019 Sulfur recovery in the sulfide-oxidizing membrane aerated biofilm reactor: experimental investigation and model simulation. Environmental Technology 40 (12), 1557–1567.

Khalil, K., Mary, B. & Renault, P. 2004 Nitrous oxide production by nitrification and denitrification in soil aggregates as affected by O2 concentration. Soil Biology and Biochemistry 36 (4), 687–699.

Kim, B., Perez-Calleja, P. & Nerenberg, R. 2020 Effect of predation on the mechanical properties and detachment of MABR biofilms. Water Research 186, 9.

Kinh, C. T., Riya, S., Hosomi, M. & Terada, A. 2017a Identification of hotspots for NO and N2O production and consumption in counter- and co-diffusion biofilms for simultaneous nitrification and denitrification. Biosource Technology 245 (August), 318–324.

Kinh, C. T., Suenaga, T., Hori, T., Riya, S., Hosomi, M., Smets, B. F. & Terada, A. 2017b Counter-diffusion biofilms have lower N2O emissions than co-diffusion biofilms during simultaneous nitrification and denitrification: insights from depth-profile analysis. Water Research 124, 363–371.

Konopka, A. 2009 What is microbial community ecology? The ISME Journal 3 (11), 1223–1230.

Kreft, J.-U. & Wimpenney, J. W. 2001 Effect of EPS on biofilm structure and function as revealed by an individual-based model of biofilm growth. Water Science and Technology 45 (6), 135–135.

Kristiansen, R., Nguyen, H. T. T., Saunders, A. M., Nielsen, J. L., Wimmer, R., Le, V. Q., McIlroy, S. J., Petrovski, S., Seviour, R. J., Calteau, A., Nielsen, K. L. & Nielsen, P. H. 2015 An metabolic model for members of the genus Tetrasphaera involved in enhanced biological phosphorus removal. The ISME Journal 7 (3), 543–554.

Kuenen, J. G. 2008 Anammox bacteria: from discovery to application. Nature Reviews Microbiology 6 (4), 520–526.

Kunetz, T. E., Oskouie, A., Poonsapaya, A., Peeters, J., Adams, N., Long, Z. & Côté, P. 2016 Innovative membrane-aerated biofilm reactor pilot test to achieve low-energy nutrient removal at the Chicago MWRD. Proceedings of the Water Environment Federation 2016 (14), 2973–2987.

Kunlaspreadee, P. & Visvanathan, C. 2020 Performance evaluation of membrane-aerated biofilm reactor for acetonitrile wastewater treatment. Journal of Environmental Engineering 146 (7), 04020055.

LaPara, T. M., Cole, A. C., Shanahan, J. W. & Semmens, M. J. 2006 The effects of organic carbon, ammoniacal-nitrogen, and oxygen partial pressure on the stratification of membrane aerated biofilms. Journal of Industrial Microbiology & Biotechnology 33 (4), 315–325.

Lackner, S., Gilbert, E. M., Vlaeminck, S. E., Joss, A., Horn, H. & van Loosdrecht, M. C. M. 2014 Full-scale partial nitritation/anammox experiences – an application survey. Water Research 55, 292–303.

Lan, M., Li, M., Liu, J., Quan, X., Li, Y. & Li, B. 2018 Coal chemical reverse osmosis concentrate treatment by membrane-aerated biofilm reactor system. Bioresource Technology 270, 120–128.

Landes, N., Rahman, A., Morse, A. & Jackson, W. A. 2021 Performance of a lab-scale membrane aerated biofilm reactor treating nitrogen dominant space-based wastewater through simultaneous nitrification-denitriﬁcation. Journal of Environmental Chemical Engineering 9 (1), 104644.

LaPara, T. M., Cole, A. C., Shanahan, J. W. & Semmens, M. J. 2006 The effects of organic carbon, ammoniacal-nitrogen, and oxygen partial pressure on the stratification of membrane aerated biofilms. Journal of Industrial Microbiology & Biotechnology 33 (4), 315–325.

Li, Q. 2018 Pilot-scale Plant Application of Membrane Aerated Biofilm Reactor (MABR) Technology in Wastewater Treatment. Master Thesis, School of Architecture and The Built Environment, KTH Royal Institute of Technology, Stockholm, Sweden.

Li, T. & Liu, J. 2019 Factors affecting performance and functional stratification of membrane-aerated biofilms with a counter-diffusion configuration. RSC Advances 9 (50), 29337–29346.

Liu, H., Yang, F., Shi, S. & Liu, X. 2010 Effect of substrate COD/N ratio on performance and microbial community structure of a membrane aerated biofilm reactor. Journal of Environmental Sciences 22 (4), 540–546.

Liu, H., Tan, S., Sheng, Z., Yu, T. & Liu, Y. 2015 Impact of oxygen on the coexistence of nitrification, denitrification, and sulfate reduction in oxygen-based membrane aerated biofilm. Canadian Journal of Microbiology 61 (3), 237–242.

Liu, Y., Ngo, H. H., Guo, W., Peng, L., Pan, Y., Guo, J., Chen, X. & Ni, B.-J. 2016 Autotrophic nitrogen removal in membrane-aerated biofilm reactors: archaeal ammonia oxidation versus bacterial ammonia oxidation. Chemical Engineering Journal 302, 535–544.

Long, Z., Houweling, D., Ireland, J., Peeters, J., Coutts, D. & Reeve 2020 ‘ZeeNAMMOXTM: Cracking the Code on Resilient and Cost-effective Side-stream Nitrogen Removal’ in Innovations in process engineering 2021. Virtual.

Longo, S., d’Antoni, B. M., Bongards, M., Chaparro, A., Cronrath, A., Fatone, F., Lema, J. M., Mauricio-Iglesias, M., Soares, A. & Hospido, A. 2016 Monitoring and diagnosis of energy consumption in wastewater treatment plants. A state of the art and applications, Chemical Engineering Journal 245–246, 302–303.

Lu, D., Bai, H., Kong, F., Liss, S. N. & Liao, B. 2020 Recent advances in membrane aerated biofilm reactors. Critical Reviews in Environmental Science and Technology.

Ma, C., Jensen, M. M., Smets, B. F. & Thamdrup, B. 2017a Pathways and controls of N2O production in nitritation–anammox biomass. Environmental Science & Technology 51 (16), 8981–8991.

Ma, Y., Domingo-Félez, C., Płosz, B. G. & Smets, B. F. 2017b Intermittent aeration suppresses nitrite-oxidizing bacteria in membrane-aerated biofilms: a model-based explanation. Environmental Science & Technology 51 (11), 6146–6155.

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

Martin, K. J., Piccioreau, C. & Nerenberg, R. 2013 Multidimensional modeling of biofilm development and fluid dynamics in a hydrogen-based, membrane biofilm reactor (MBR). Water Research 47 (13), 4739–4751.
Martin, K., Sathyamoorthy, S., Houweling, D., Long, Z., Peeters, J. & Snowling, S. 2017 A sensitivity analysis of model parameters influencing the biofilm nitrification rate: comparison between the aerated biofilm reactor (MABR) and integrated fixed film activated sludge (IFAS) process. *Proceedings of the Water Environment Federation* 2017 (16), 257–265.

McLamore, E. S., Zhang, W., Porterfield, D. M. & Banks, M. K. 2010 Membrane-aerated biofilm proton and oxygen flux during chemical toxin exposure. *Environmental Science & Technology* 44 (18), 7050–7057.

Mehrabi, S., Houweling, D. & Dagnew, M. 2020 Establishing mainstream nitrite shunt process in membrane aerated biofilm reactors: impact of organic carbon and biofilm scouring intensity. *Journal of Water Process Engineering* 37, 101460.

Mehrabi, S., Houweling, D. & Dagnew, M. 2021 Single-Stage Biofilm-Based Total Nitrogen Removal in a Membrane Aerated Biofilm Reactor: Impact of Aeration Mode, HRT and Scouring Intensity, In Review. Available from: https://www.researchsquare.com/article/rs-400202/v1 (accessed 25 May 2021).

Mei, X., Liu, J., Guo, Z., Li, P., Bi, S., Wang, Y., Yang, Y., Shen, W., Wang, Y., Xiao, Y., Yang, X., Zhou, B., Liu, H. & Wu, S. 2019 Simultaneous p-nitrophenol and nitrogen removal in PNP wastewater treatment: comparison of two integrated membrane-aerated bioreactor systems. *Journal of Hazardous Materials* 363 (September 2018), 99–108.

Misiak, K., Casey, E. & Murphy, C. D. 2011 Factors influencing 4-fluorobenzoate degradation in biofilm cultures of Pseudomonas knackmussii B13. *Water Research* 45 (11), 3512–3520.

Mooshammer, M., Kitzinger, K., Schintlmeister, A., Ahmerkamp, S., Nielsen, J. L., Nielsen, P. H. & Wagner, M. 2021 Flow-through stable isotope probing (Flow-SIP) minimizes cross-feeding in complex microbial communities. *The ISME Journal* 15 (1), 348–353.

Morley, N., Baggs, E. M., Dörsch, P. & Bakken, L. 2008 Production of NO, N2O and N2 by extracted soil bacteria, regulation by NO2– and O2 concentrations: production of NO, N2O and N2 by extracted soil bacteria. *FEMS Microbiology Ecology* 651, 102–112.

Morse, A., Jackson, W. A. & Karparthi, S. 2004 Biological Treatment of A Urine-Humidity Condensate Waste Stream.

Nathan, N., Shefer, I., Shechter, R., Sisso, Y. & Gordon, K. J. 2020 Start-up of A Full-Scale Activated Sludge Retrofit Using A Spirally-Wound MABR – Results and Model Evaluation.

Nerenberg, R. 2016 The membrane-biofilm reactor (MBfR) as a counter-diffusional biofilm process. *Current Opinion in Biotechnology* 38, 131–136.

Newhart, K. B., Holloway, R. W., Hering, A. S. & Cath, T. Y. 2019 Data-driven performance analyses of wastewater treatment plants: a review. *Water Research* 157, 498–513.

Nielsen, P. H., Kruglund, C., Seviour, R. J. & Nielsen, J. L. 2009 Identity and ecophysiology of filamentous bacteria in activated sludge. *FEMS Microbiology Reviews* 33 (6), 969–998.

Paepe, J. D., Paepe, K. D., Godja, F. & Rabaeck, K. 2020 Bio-electrochemical COD removal for energy-efficient, maximum and robust nitrogen recovery from urine through membrane aerated nitrification. *Water Research* 185, 116223.

Peeters, J., Long, Z., Houweling, D., Côté, P., Daigger, G. T. & Snowling, S. 2017a Nutrient removal intensification with MABR – developing a process model supported by piloting. *Proceedings of the Water Environment Federation* 2017 (5), 657–669.

Peeters, J., Adams, N., Long, Z., Côté, P. & Kunetz, T. 2017b Demonstration of innovative MABR low-energy nutrient removal technology at Chicago MWRD. *Water Practice and Technology* 12 (4), 927–936.

Pellicer-Nàcher, C., Sun, S., Lackner, S., Terada, A., Schreiber, F., Zhou, Q. & Smets, B. F. 2010 Sequential aeration of membrane-aerated biofilm reactors for high-rate autotrophic nitrogen removal: experimental demonstration. *Environmental Science & Technology* 44 (19), 7628–7634.

Pellicer-Nàcher, C., Domingo-Félez, C., Lackner, S. & Smets, B. F. 2013 Microbial activity catalyzes oxygen transfer in membrane-aerated nitritating biofilm reactors. *Journal of Membrane Science* 446, 465–471.

Pellicer-Nàcher, C., Franck, S., Gülay, A., Ruscalleda, M., Terada, A., Al-Soud, W. A., Hansen, M. A., Sørensen, S. J. & Smets, B. F. 2014 Sequentially aerated membrane biofilm reactors for autotrophic nitrogen removal: microbial community composition and dynamics. *Microbial Biotechnology* 7 (1), 32–43.

Perez-Calleja, P., Aybar, M., Piccoreanu, C., Esteban-Garcia, A. L., Martin, K. J. & Nerenberg, R. 2017 Periodic venting of MABR lumen allows high removal rates and high gas transfer efficiencies. *Water Research* 121, 349–360.

Petrova, O. E. & Sauer, K. 2016 Escaping the biofilm in more than one way: desorption, detachment or dispersion. *Current Opinion in Microbiology* 30, 67–78.

Plascencia-Jatomea, R., Almazán-Ruiz, F. J., Gómez, J., Rivero, E. P., Monroy, O. & González, I. 2015 Hydrodynamic study of a novel membrane aerated biofilm reactor (MABR): tracer experiments and CFD simulation. *Chemical Engineering Science* 138, 324–332.

Qi, W. K., Guo, Y. L., Xue, M. & Li, Y. Y. 2013 Hydraulic analysis of an upflow sand filter: tracer experiments, mathematical model and CFD computation. *Chemical Engineering Science* 104, 460–472.

Quan, X., Huang, K., Li, M., Lan, M. & Li, B. 2018 Nitrogen removal performance of municipal reverse osmosis concentrate with low C/N ratio by membrane-aerated biofilm reactor. *Frontiers of Environmental Science & Engineering* 12 (6), 5.

Rittmann, B. E., Boltz, J. P., Brockmann, D., Daigger, G. T., Morgenroth, E., Sørensen, K. H., Tkáč, I., Van Loosdrecht, M. & Vanrolleghem, P. A. 2018 A framework for good biofilm reactor modeling practice (GBRMP). *Water Science and Technology* 77 (5), 1149–1164.

Rosso, D., Larson, L. E. & Stenstrom, M. K. 2008 Aeration of large-scale municipal wastewater treatment plants: state of the art. *Water Science and Technology* 57 (7), 975–978.

Sabba, F. 2018 Nitrous oxide emissions from biofilm processes for wastewater treatment. *Applied Microbiology and Biotechnology* 15.
Tian, H., Xu, X., Qu, J., Li, H., Hu, Y., Huang, L., He, W. & Li, B. 2020 Biodegradation of phenolic compounds in high saline wastewater by biofilms adhering on aerated membranes. *Journal of Hazardous Materials* **392**, 122463.

Underwood, A., McMains, C., Coutts, D., Peeters, J., Ireland, J. & Houweling, D. 2018 Design and startup of the first full-scale membrane aerated biofilm reactor in the United States. *Proceedings of the Water Environment Federation* **2018** (16), 1282–1296.

Uri, N., Constantine, T., Sandino, J., Willoughby, A. & Nielsen, P. H. 2018 Membrane-aerated biofilm reactor (MABR) demonstration at Ejby Mølle WRFF. *Proceedings of the Water Environment Federation* **2018** (5), 201–207.

Uri-Carreno, N., Nielsen, P. H., Gernaey, K. & Flores-Alsina, X. 2021 Long-term operation assessment of a full-scale membrane-aerated biofilm reactor under Nordic conditions. 779.

Wang, J., Liu, G. F., Lu, H., Jin, R. F., Zhou, J. T. & Lei, T. M. 2012 Biodegradation of Acid Orange 7 and its auto-oxidative decolorization product in membrane-aerated biofilm reactor. *International Biodeterioration and Biodegradation* **67**, 73–77.

Wang, R., Xiao, F., Wang, Y. & Lewandowski, Z. 2016 Determining the optimal transmembrane gas pressure for nitrification in membrane-aerated biofilm reactors based on oxygen profile analysis. *Applied Microbiology and Biotechnology* **100**(17), 7699–7711.

Wanner, O., Eberl, H. J., Morgenroth, B., Noguera, D. R., Picioreanu, C., Rittmann, B. E. & Loosdrecht, M. C. M. 2006 Mathematical Modeling of Biofilms.

Wei, X., Li, B., Zhao, S., Wang, L., Zhang, H., Li, C. & Wang, S. 2012 Mixed pharmaceutical wastewater treatment by integrated membrane-aerated biofilm reactor (MABR) system – a pilot-scale study. *Bioresource Technology* **122**, 189–195.

Wu, Y., Wu, Z., Chu, H., Li, J., Hao, H., Guo, W., Zhang, N. & Zhang, H. 2019 Comparison study on the performance of two different gas-permeable membranes used in a membrane-aerated biofilm reactor. *Science of the Total Environment* **658**, 1219–1227.

Xiao, P., Zhou, J., Luo, X., Kang, B., Guo, L., Yuan, G., Zhang, L. & Zhao, T. 2021 Enhanced nitrogen removal from high-strength ammonium wastewater by improving heterotrophic nitrification-aerobic denitrification process: insight into the influence of dissolved oxygen in the outer layer of the biofilm. *Journal of Cleaner Production* **297**, 126658.

Xu, F., Cao, F., Kong, Q., Zhou, L., Yuan, Q., Zhu, Y., Wang, Q., Du, Y. & Wang, Z. 2018 Electricity production and evolution of microbial community in the constructed wetland-microbial fuel cell. *Chemical Engineering Journal* **339**, 479–486.

Zeng, J. & Liu, J. 2015 Economic model predictive control of wastewater treatment processes. *Industrial & Engineering Chemistry Research* **54**(21), 5710–5721.

Zhang, H., Gong, W., Zeng, W., Chen, R., Lin, D., Li, G. & Liang, H. 2021 Bacterial-algae biofilm enhance MABR adapting a wider COD/N ratios wastewater: performance and mechanism. *Science of The Total Environment* **781**, 146663.

Zhao, L., Dai, T., Qiao, Z., Sun, P., Hao, J. & Yang, Y. 2020 Application of artificial intelligence to wastewater treatment: a bibliometric analysis and systematic review of technology, economy, management, and wastewater reuse. *Process Safety and Environmental Protection* **135**, 169–182.

Zhao, B., Ma, X., Xie, F., Cui, Y., Zhang, X. & Yue, X. 2021 Development of simultaneous nitrification-denitrification and anammox and in-situ analysis of microbial structure in a novel plug-flow membrane-aerated sludge blanket. *Science of The Total Environment* **750**, 142296.

Zhong, H., Wang, H., Tian, Y., Liu, X., Yang, Y., Zhu, L., Yan, S. & Liu, G. 2019 Treatment of polluted surface water with nylon silk carrier-aerated biofilm reactor (CABR). *Bioresource Technology* **289**, 121617.

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