Municipal Wastewater Treatment Using Membrane Aerated Biofilm Reactor (MABR): Mechanisms, Performance and Microbial Community

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
in
Environmental Engineering

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ABSTRACT

The current drive for energy neutrality, drastic reduction in energy costs and increasingly stringent wastewater discharge rules has led to the quest for the development of more energy efficient and effective wastewater treatment technologies. MABR is one of these developing technologies. MABR is a biofilm-based technology that offer improved performance with regards to pollutants removal from wastewater, energy efficiency and environmental sustainability. Currently, MABR is garnering wide acceptance due to process and operational advantages that have accrued during many years of investigation. But despite these achievements, the challenge of MABR biofilm thickness management still persists.

Additional benefits in terms of energy efficiency and savings in operational costs for wastewater treatment could also be realized by combining the MABR advantages with nitritation. However, the range of operational factors and substrate conditions, that can be manipulated to suppress NOB for stable nitritation are absent both in MABR and mainstream wastewater. Consequently, for the treatment of conventionally collected sewage, establishing nitritation in MABR is challenging. This work was therefore designed to expand the current knowledge of MABR operations, particularly with respect to biofilm thickness management, performance stability and the establishment of nitritation in MABR for municipal wastewater treatment. Municipal wastewater remains one of the largest sources of environmental degradation as well as eutrophication of receiving water bodies. Eutrophication alters waterbody ecosystems equilibrium, leads to secondary water pollution and limits the possibilities of wastewater reuse.

In this study, two parallel MABRs; R1 and R2 were operated continuously for more than 250 days, using real and synthetic wastewaters. R1 was a municipal wastewater system, applied in the optimization of MABR performance, study of structural and microbial community dynamics

analysis of MABR biofilm, and the potential of the MABR to perform stable nitritation under mainstream conditions. While R2 was a high strength synthetic wastewater system, applied in the study of performance and stability of MABR under high strength wastewater conditions and the impact of inoculum on MABR performance and microbial community ecology.

To optimize the performance of MABR for real municipal wastewater treatment, graduated HRT was applied with biomass recirculation until organic carbon, ammonia nitrogen and total nitrogen removal efficiencies reached 98, 96 and 67% respectively with acceptable effluent quality at a low HRT of 3h and operating pressure of 2psia.

For MABR biofilm management and performance stability study, a protocol involving biomass recirculation and intermittent membrane cleaning induced by a drop in DO to set-point of 0.2 mg/L was developed. When applied in the treatment of municipal wastewater, the MABR demonstrated average organic carbon, ammonia nitrogen and total inorganic nitrogen removal efficiencies of 92 ± 2%, 100 ± 7.8% and 84 ± 5% respectively, at mean surface loading rates of 10 ± 0.7 gCOD/m2/d and 0.93 ± 0.07 gN/m^2/d within a low hydraulic retention time of 2.5 h. Microbial population at each stage of investigation indicated sufficient biodiversity and relative abundance for stable reactor performance.

The potentiality of nitritation development and sustenance in MABR for mainstream wastewater treatment was also investigated. This aspect of the study was accomplished in four phases using a combination of continuous and intermittent aeration modes with aerated and non-aerated cycles of 10 (5 on: 5 off), 20 (10 on: 10 off) and 25 (10 on: 15 off) minutes respectively, and a constant hydraulic retention time (HRT) of 2.5h. Nitrite accumulation rate (NAR), nitrate production rate (NPR) and ammonium nitrogen removal efficiency (ANRE) achieved in Phases II - IV were, 35%, 12% and 99%; 76%, 3.4% and 98%; 94%, 1% and 98% respectively. Between the

initiation of intermittent aeration and termination of the study, ammonia oxidizing bacteria (AOB) activities within the reactor increased by >150%. In contrast, NOB activities declined by >60 %.

Finally, the impact of combined inocula on MABR biofilm properties was explored in a 4-stage study using HRTs of 24, 10, 6 and 4h for high strength wastewater treatment. Microscopy analysis of the biofilm at stage four of the study using; cells viability analysis, SEM and TEM revealed low viable cells and low biofilm concentration on membrane surface. Biofilm thickness was determined to be 0.357mm, thus simultaneous nitrification-denitrification was inhibited, leading to poor total inorganic nitrogen removal. Mean COD removal efficiency was estimated to be >80% while the mean removal efficiencies for $NH4^{+}$–N and TIN were 60 and 13 % respectively. Further investigations are required to collaborate these results and optimize the study parameters.

PREFACE

The contents of this thesis are my original work under supervision of Dr. Yang Liu. Dr. Liu contributed significantly to every aspect of this work. Other colleagues that contributed to this research are listed below.

Yun Zhou contributed to the investigation in chapter 3 while Md Shaheen contributed to the microbial analysis of same Chapter.

Korris Lee contributed in editing of Chapter 4, while Yingdi Zhang analyzed the microbial community of Chapters 4 and 5.

Finally, versions of Chapter 3, 4 and 5 have been published in journals while Chapter 6 is currently under review for journal publication.

DEDICATION

To my beloved parents;

Mr. Joachim Uwadiegwu and Ugodiya Christiana Nkechi Ogbuagu

Pioneers of "*Access to Education*" in Oyibo community, Rivers State, Nigeria.

Your service to humanity is eternally appreciated.

And my beloved children; Akwaugo, Jideofor and Okeoma;

thank you for helping me uphold "*The Ukaigwe Brand"*

Dim,

Alford Kenechukwu Ukaigwe

My anchor in every situation*; Your project is now complete*!

ACKNOWLEDGEMENT

My deepest and heartfelt gratitude goes to my indefatigable supervisor, Dr. Yang Liu for giving me the opportunity to work on this project. Thank you for your supervision, patience and support throughout this program. Sincere appreciation also goes to my supervisory and candidacy examination committee members; Dr. Ian Buchanan, Dr. Wenming Zhang, Dr. Bipro Dhar, Dr. Jeff Farner, Dr. Jinfeng Liu and Dr. Lijun Deng for their guidance and insightful comments before and during the candidacy examination.

To the past and present members of Dr. Yang Liu's Research Group; Dr. Oliver Iorhemen, Dr. Yun Zhou, Dr. Ahmed Sharaf, Dr. Mian Nabeel Anwar, Xin Zou and the other wonderful members of the group too numerous to mention, the diversity of perspectives and knowledge within our group made working in the team a pleasure, special thanks to you all.

The unconditional love I received from my family and friends is also gratefully acknowledged. Thank you all for supporting and standing by me through every challenge and triumph.

And finally, unto the King eternal, immortal, invisible, the only wise God, be honour and glory for ever and ever. Amen.

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NOMENCLATURE

MABR	Membrane aerated biofilm reactor
ASV	amplicon sequence variants
AGS	Aerobic granular sludge
AOB	Ammonia oxidizing bacteria
ANRE	Ammonium nitrogen removal efficiency
AnAOB	Anaerobic ammonia oxidizing bacteria
Anammox	Anaerobic ammonium oxidation
Comammox	Complete ammonia oxidation
CANDO	Coupled aerobic-anoxic nitrous decomposition operation
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
DND	Denitrifying bacteria
DO	Dissolved oxygen
DNPAOs	Denitrifying polyphosphate-accumulating organisms
EPS	Extracellular polymeric substances
GHG	Greenhouse gas
NPR	Nitrate production rate
NAR	Nitrite accumulation rate
NOB	Nitrite oxidizing bacteria
EPS	Extracellular polymeric substances
HRT	Hydraulic retention time

SEM	Scanning electron microscope
SRT	Solids retention time
SLR	Surface loading rate
SSND	Single-stage simultaneous nitrification-denitrification
OTE	Oxygen transfer efficiency
OTR	Oxygen transfer rate
PN/A	Partial nitrification /anammox
PND	Partial nitrification-denitrification
PVDF	Polyvinylidene difluoride
PRE	Pollutants removal efficiency
TEM	Transmission electron microscope
TN	Total nitrogen
TIN	Total inorganic nitrogen

Chapter 1 - Introduction

1.1. Background

Biological wastewater treatment is generally energy intensive, and aeration constitutes over 50% of this energy use (USEPA, 1999). Traditionally, wastewater has been treated biologically using the conventional activated sludge (CAS) system, a system that employ diffusers for oxygen transfer into wastewater during treatment process, but diffuser have low oxygen transfer efficiency (Metcalf, Eddy et al. 2014).

The MABR is a contemporary technology for wastewater treatment based on direct oxygen supply into wastewater treatment. It combines the advantages of fixed-biofilm process and direct oxygen supply into wastewater using bubble-free aeration (Syron and Casey 2008, Martin, Boltz and Nerenberg 2012). Moreover, it uses counter-diffusive electron transfer mechanism which offer operational advantages over traditional wastewater treatment and conventional biofilm processes, offering more than 4 times higher performance with regards to oxygen transfer rate and transfer efficiency (Aybar, Pizarro et al. 2014, Metcalf, Eddy et al. 2014, Nerenberg 2016). The MABR technology has been a subject of extensive research for over three decades and numerous studies have demonstrated the potential of the technology to reduce the energy required for biological wastewater treatment in addition to other benefits (Syron and Casey 2008, Kunetz, Oskouie et al. 2016, Nerenberg 2016, Houweling, Peeters et al. 2017, Sunner, Long et al. 2018).

In the MABR system, pollutant oxidation occur in the biofilm, which grows on the membrane surface, oxygen is supplied into the biofilm through the membrane cavity (Syron and Casey 2008, Ukaigwe, Zhou et al. 2021), this mechanism eliminates oxygen wastage and can produce up to 100% oxygen transfer efficiency (OTE) (Aybar, Pizarro et al. 2014, Perez-Calleja, Aybar et al. 2017). Oxygen from membrane cavity and substrates from wastewater flow into the

biofilm from opposite directions, creating a unique counter diffusion profile. Counter diffusion flow results in a 3-region habitat (aerobic, anoxic and anaerobic) with highly diverse microbial community within the biofilm (Downing and Nerenberg 2008, Sun, Nàcher et al. 2010).

MABR also offer improved performance with regards to removal of nutrients (nitrogen and phosphorus) from wastewater due to its efficient mass transfer capacity, multi-population microbial community that provides multiple functionalities. Additionally, the stratification within its biofilm provides protection to the different microbial groups within the system. (Sun, Nàcher et al. 2010, Syron, Semmens and Casey 2015, Underwood, McMains et al. 2018, Long, Oskouie et al. 2020). However, the efficiency of the mass transfer operation and the degree of the microbial diversity is dependent on the thickness of the biofilm. MABR biofilm thickness control the entirety of the process, excessive biofilm growth can weaken the process performance and in extreme situations disrupt the MABR operation (Hou, Jassby et al. 2019, Sanchez-Huerta, Fortunato et al. 2022), but controlling this biofilm thickness is challenging. Biofilm development dynamics has been extensively studied, yet managing MABR biofilm thickness in long term operations still remains a constraining issue (Wagner, Daigger and Love 2022).

Traditionally, nitrogen removal from wastewater is through the nitrification pathway, however, the nitritation pathway is considered more energy efficient and sustainable in comparison, due to lower oxygen and organic carbon requirement as a result of nitrate bypass and reduced footprint (Pellicer-Nàcher, Sun et al. 2010, Regmi, Miller et al. 2014, Wang, Xu et al. 2019, Liu, Kim et al. 2020, Zhao, Guo et al. 2022). The key to stable nitritation in MABR for long-term operation is to ensure the sustenance of NOB inhibiting activities through careful control of process parameters while the activity of AOB and denitrifiers are unhindered (Wang, Xu et al. 2019, Wang, Liang et al. 2021). Studies have shown that establishing and maintaining nitritation in MABR can

be challenging as MABR configuration potentially interfere with NOB inhibition (Ma, Piscedda et al. 2022), but it is still achievable because the MABR system can be manipulated to maintain conditions adequate for the inhibition of NOB activities for instance low DO milieu can be maintained through intermittent aeration (Pellicer-Nàcher, Sun et al. 2010, Ma, Domingo-Felez et al. 2017). Bunse et al., 2020 and Chen et al., 2022, both successfully demonstrated the feasibility of nitritation in MABR at mainstream conditions using intermittent aeration.

Real and synthetic wastewaters were used in this study to investigate the processes, the biofilm and the microbial community dynamics in an MABR for municipal wastewater treatment.

1.2. Problem statement

Water is integral to healthy ecosystems and human survival, serving as the crucial link between the climate system, human society and the environment. But trends such as, climate change, increasing world population, changes in farming practices, among other factors, have caused high rise in water consumption as well as significant deterioration in its quality. Consequently, higher restrictive limits are being imposed on municipal wastewater effluents, necessitating the need to improve existing wastewater treatment facilities and to develop more advanced wastewater treatment technologies, in order to comply with this new effluent discharge limits. Water quality is affected the most by organic matter and nutrients content (phosphorus and nitrogen) in water bodies. Presently, the quantity of global municipal wastewater with an average nitrogen concentration of 40 mg/L has already hit 380 billion m^3/year, and will continue to increase due to rapid global urbanization and increasing population (Qadir, Drechsel et al. 2020) but water bodies have low threshold nutrient content (Dodds and Welch 2000) and thus, the urgent need for nitrogen removal from wastewater.

Numerous studies have demonstrated the capacity of MABR to remove organic matter and nutrients from municipal wastewater, reduce volatile gas emission and produce high quality effluent at significantly reduced aeration energy costs and smaller footprint in comparison to the conventional activated sludge (CAS) treatment process. MABR operational process can also be designed to produce alternate aerobic and anoxic conditions within the system, in order to inhibit NOB activities and also prevent its adaptation to low DO condition. This will ensure the long-term suppression of NOB activities and removal of nitrogen removal via the nitritation pathway. Additionally, the MABR-Nitritation nexus will represents an ultra-efficient wastewater treatment pathway that combines the advantages of two efficient processes; biofilm and nitritation processes.

Unfortunately, despite the achievements and potentials of the MABR system, limitations both in operational application and process parameters that require significant research still abound (Martin, Boltz and Nerenberg 2012, Nerenberg 2016, Lema and Martinez 2017). For instance, MABR biofilm characteristics control the success of the entire MABR system, and managing MABR biofilm condition to be adequate for specific purposes is still challenging. Biofilm thickness is a typical performance indicator of the MABR technology and its control remains a barrier to the widespread application of the technology (Suarez, Piculell et al. 2019, He, Wagner et al. 2021).

Furthermore, the development and maintenance of nitritation in MABR for the treatment of mainstream wastewater is also challenging because nitritation-supporting conditions inherent in other biological systems and wastewater types are lacking in both the MABR and conventionally collected sewage. Moreover, very limited research has been conducted on nitritation in MABR for mainstream wastewater treatment. Therefore, to establish a sustainable nitritation process in MABR remains challenging and require more research to address specific knowledge gaps.

In response to the above challenges and to bridge the research gaps in the MABR technology development, we studied the MABR system, developed a biofilm thickness management protocol that ensured the maintenance of biofilms with adequate thickness to sustain MABR operations for extended operation, with high performance at high substrate loadings. We also investigated the impact of intermittent aeration on the development and sustenance of nitritation in MABR. MABR displayed promising results for nitrogen removal through nitritation under mainstream conditions.

1.3. Study objectives

The overall objective of work is to study conventional MABR operations for the treatment of mainstream wastewater, with respect to process parameter optimization, biofilm thickness management, nitrogen removal pathways and microbial community dynamics. The goal is to expand the current knowledge on biofilm thickness management strategies and establish nitritation in traditional MABR systems for long-term operation. Two key areas critical to the advancement of the MABR technology, which continues to present significant challenges to MABR operations. This objective is subdivided into 3 parts, each with specific objectives:

1. **Operate and optimize an MABR for the treatment of conventionally collected sewage.**

   Hypothesis: The high biomass concentration of the MABR biofilm can offset, the risk of low HRT operation, thereby sustaining MABR performance and stability over an extended period.

   Specific objectives:

   a.) Explore the impact of high and variable loads on the performance, stability and microbial community structure of traditional MABR. Optimize HRT.

   b.) Study the impact of HRT on the microbial community structure

2. **Study the structure, functional characteristics and microbial community dynamics of the MABR biofilms and their responses to operational conditions and parameter changes.**

Hypothesis: With careful control of bulk dissolved oxygen (DO) and biofilm thickness through non-aggressive cleaning, biofilm of appropriate thickness could be sustained in the MABR which will enable efficient organic carbon, ammonia, and nitrite oxidations at high surface loading rates while simultaneously sustaining denitrification.

Specific objectives:

a.) Develop biofilm control strategies for improved MABR performance

b.) Evaluate the impact of the thickness control strategy on biofilm properties, process stability, performance and microbial community structure

3. **Develop DO-based control strategy for energy efficient nitritation in MABR**

Hypothesis: with a stable nitrifying biofilm within the MABR, careful control of system DO through intermittent aeration can convert the biofilm from nitrifying to nitritating, where AOBs continuous to flourish, and NOBs sufficiently suppressed for long term stable nitritation.

Specific objectives:

a.) Develop and evaluate aeration strategy to sustain nitritation in MABR

b.) optimize the aerated and non-aerated cycle durations for nitritation

c.) Evaluate the MABR performance and correlate the microbial community structure with process operating conditions.

1.4. Thesis organization

This thesis consists of seven chapters, arranged in a manuscript format. Introduction, experimental procedures, results, and discussions are presented separately in each chapter (Chapters 3-6). However, the generic parts in materials and methods were not repeated.

A brief introduction to MABR, its potentials, advantages and challenges as well as problem statement, the research objectives and the thesis organization are presented in Chapter 1.

Literature review covering; overview of biological wastewater treatment and overview of MABR application in biological wastewater treatment, principles of the MABR technology**,** design and operation considerations, MABR membrane, biofilms, operation strategy, performance, nitrogen removal pathways and energy efficiency were covered in Chapter 2.

Chapter 3 is the preliminary stage of this research project. Here, the performance of an MABR treating real municipal wastewater primary effluent was presented. The performance of the MABR in terms of pollutants removal efficiency and stability under variable loading rates were tested in order to optimize the process HRT.

MABR biofilm structures, properties, microbial community, thickness control mechanisms and process stability were presented in Chapter 4. Biofilm thickness and microbial community relative abundance over the course of the study period were quantified, in order to assess the impact of the thickness control strategy developed on the process parameters and the result presented in this chapter.

The impact of intermittent aeration on the development and sustenance of nitritation in MABR for the treatment of municipal wastewater was the focus of Chapter 5. Biofilm development and the impact of intermittent aeration on the activities of nitrifiers were also covered in this chapter.

In Chapter 6, the impact of combined inocula on MABR biofilm development dynamics, microbial community structure and MABR performance was presented.

Conclusions from Chapters 3-6 and recommendations for future research were presented in Chapter 7.

1.5. References

USEPA, 1999. Wastewater technology fact sheet, fine bubble aeration, EPA 832-F-99-065. U.S. Environmental Protection Agency: EPA Office of Water. Washington DC.

Aybar, M., G. Pizarro, J. Boltz, L. Downing and R. Nerenberg (2014). "Energy-efficient wastewater treatment via the air-based, hybrid membrane biofilm reactor (hybrid MfBR)." Water science and technology **69**(8): 1735-1741.

Boltz, J. and G. Daigger (2010). "Uncertainty in bulk-liquid hydrodynamics and biofilm dynamics creates uncertainties in biofilm reactor design." Water Science and Technology **61**(2): 307-316.

Boltz, J. P., B. F. Smets, B. E. Rittmann, M. C. Van Loosdrecht, E. Morgenroth and G. T. Daigger (2017). "From biofilm ecology to reactors: a focused review." Water Science and Technology **75**(8): 1753-1760.

Bunse, P., L. Orschler, S. Agrawal and S. Lackner (2020). "Membrane aerated biofilm reactors for mainstream partial nitritation/anammox: Experiences using real municipal wastewater." Water Research X **9**: 100066.

Castrillo, M., R. Díez-Montero, A. L. Esteban-García and I. Tejero (2019). "Mass transfer enhancement and improved nitrification in MABR through specific membrane configuration." Water research **152**: 1-11.

Chen, R., S. Cao, L. Zhang and Y. Zhou (2022). "NOB suppression strategies in a mainstream membrane aerated biofilm reactor under exceptionally low lumen pressure." Chemosphere **290**: 133386.

Dodds, W. K. and E. B. Welch (2000). "Establishing nutrient criteria in streams." Journal of the North American Benthological Society **19**(1): 186-196.

Downing, L. S. and R. Nerenberg (2008). "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.

He, H., B. M. Wagner, A. L. Carlson, C. Yang and G. T. Daigger (2021). "Recent progress using membrane aerated biofilm reactors for wastewater treatment." Water Science and Technology **84**(9): 2131-2157.

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

Houweling, D., J. Peeters, P. Cote, Z. Long and N. Adams (2017). "Proving membrane aerated biofilm reactor (MABR) performance and reliability: Results from four pilots and a full-scale plant." Proceedings of the Water Environment Federation **2017**(16): 272-284.

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

Lema, J. M. and S. S. Martinez (2017). Innovative wastewater treatment & resource recovery technologies: impacts on energy, economy and environment, IWA publishing.

Li, X., D. Bao, Y. Zhang, W. Xu, C. Zhang, H. Yang, Q. Ru, Y.-f. Wang, H. Ma and E. Zhu (2023). "Development and application of membrane aerated biofilm reactor (MABR)—A review." Water **15**(3): 436.

Liu, X., M. Kim, G. Nakhla, M. Andalib and Y. Fang (2020). "Partial nitrification-reactor configurations, and operational conditions: Performance analysis." Journal of Environmental Chemical Engineering **8**(4): 103984.

Long, Z., A. K. Oskouie, T. E. Kunetz, J. Peeters, N. Adams and D. Houweling (2020). "Simulation of long-term performance of an innovative membrane-aerated biofilm reactor." Journal of Environmental Engineering **146**(6): 04020041.

Luo, Y.-H., R. Chen, L.-L. Wen, F. Meng, Y. Zhang, C.-Y. Lai, B. E. Rittmann, H.-P. Zhao and P. Zheng (2015). "Complete perchlorate reduction using methane as the sole electron donor and carbon source." Environmental science & technology **49**(4): 2341-2349.

Ma, Y., C. Domingo-Felez, B. G. Plósz and B. F. Smets (2017). "Intermittent aeration suppresses nitrite-oxidizing bacteria in membrane-aerated biofilms: a model-based explanation." Environmental science & technology **51**(11): 6146-6155.

Ma, Y., A. Piscedda, A. D. L. C. Veras, C. Domingo-Félez and B. F. Smets (2022). "Intermittent aeration to regulate microbial activities in membrane-aerated biofilm reactors: Energy-efficient nitrogen removal and low nitrous oxide emission." Chemical Engineering Journal **433**: 133630.

Martin, K. J., J. P. Boltz and R. Nerenberg (2012). The Membrane Biofilm Reactor (MBfR) for Wastewater Treatment: Applications, Design Considerations, and Technology Outlook. Proceedings of 85th Annual Water Environment Federation Technical Exposition & Conference.

Metcalf, Eddy, M. Abu-Orf, G. Bowden, F. L. Burton, W. Pfrang, H. D. Stensel, G. Tchobanoglous, R. Tsuchihashi and AECOM (2014). Wastewater engineering: treatment and resource recovery, McGraw Hill Education.

Nerenberg, R. (2016). "The membrane-biofilm reactor (MBfR) as a counter-diffusional biofilm process." Current opinion in biotechnology **38**: 131-136.

Peeters, J., Z. Long, D. Houweling, P. Côté, G. T. Daigger and S. Snowling (2017). Nutrient removal intensification with MABR–developing a process model supported by piloting. Nutrient Removal and Recovery Symposium 2017, Water Environment Federation.

Pellicer-Nàcher, C. and B. F. Smets (2014). "Structure, composition, and strength of nitrifying membrane-aerated biofilms." water research **57**: 151-161.

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

Perez-Calleja, P., M. Aybar, C. Picioreanu, A. L. Esteban-Garcia, K. J. Martin and R. Nerenberg (2017). "Periodic venting of MABR lumen allows high removal rates and high gas-transfer efficiencies." Water research **121**: 349-360.

Pérez-Calleja, P., E. Clements and R. Nerenberg (2022). "Enhancing ammonium oxidation fluxes and nitritation efficiencies in MABRs: a modeling study." Environmental Science: Water Research & Technology **8**(2): 358-374.

Qadir, M., P. Drechsel, B. Jiménez Cisneros, Y. Kim, A. Pramanik, P. Mehta and O. Olaniyan (2020). Global and regional potential of wastewater as a water, nutrient and energy source. Natural resources forum, Wiley Online Library.

Sanchez-Huerta, C., L. Fortunato, T. Leiknes and P.-Y. Hong (2022). "Influence of biofilm thickness on the removal of thirteen different organic micropollutants via a Membrane Aerated Biofilm Reactor (MABR)." Journal of Hazardous Materials **432**: 128698.

Suarez, C., M. Piculell, O. Modin, S. Langenheder, F. Persson and M. Hermansson (2019). "Thickness determines microbial community structure and function in nitrifying biofilms via deterministic assembly." Scientific reports **9**(1): 1-10.

Sun, L., Z. Wang, X. Wei, P. Li, H. Zhang, M. Li, B. Li and S. Wang (2015). "Enhanced biological nitrogen and phosphorus removal using sequencing batch membrane-aerated biofilm reactor." Chemical Engineering Science **135**: 559-565.

Sun, S.-P., C. P. i. Nàcher, B. Merkey, Q. Zhou, S.-Q. Xia, D.-H. Yang, J.-H. Sun and B. F. Smets (2010). "Effective biological nitrogen removal treatment processes for domestic wastewaters with low C/N ratios: a review." Environmental Engineering Science **27**(2): 111-126.

Sunner, N., Z. Long, D. Houweling, A. Monti and J. Peeters (2018). MABR as a low-energy compact solution for nutrient removal upgrades—results from a demonstration in the UK. WEFTEC 2018, Water Environment Federation.

Syron, E. and E. Casey (2008). "Membrane-aerated biofilms for high rate biotreatment: performance appraisal, engineering principles, scale-up, and development requirements." Environmental science & technology **42**(6): 1833-1844.

Syron, E. and B. Heffernan (2017). OxyMem, The Flexiable MABR. Nutrient Removal and Recovery Symposium 2017, Water Environment Federation.

Syron, E., M. J. Semmens and E. Casey (2015). "Performance analysis of a pilot-scale membrane aerated biofilm reactor for the treatment of landfill leachate." Chemical Engineering Journal **273**: 120-129.

Terada, A., T. Yamamoto, R. Igarashi, S. Tsuneda and A. Hirata (2006). "Feasibility of a membrane-aerated biofilm reactor to achieve controllable nitrification." Biochemical engineering journal **28**(2): 123-130.

Tian, H.-L., J.-Y. Zhao, H.-Y. Zhang, C.-Q. Chi, B.-A. Li and X.-L. Wu (2015). "Bacterial community shift along with the changes in operational conditions in a membrane-aerated biofilm reactor." Applied microbiology and biotechnology **99**: 3279-3290.

Tian, H., M. Hui, P. Pan, J. Huang, L. Chen and J. Zhao (2021). "Performance and microbial ecology of biofilms adhering on aerated membrane with distinctive conditions for the treatment of domestic sewage." Environmental Technology **42**(3): 459-467.

Ukaigwe, S., Y. Zhou, M. Shaheen and Y. Liu (2021). "Municipal wastewater treatment using a membrane aerated biofilm reactor." Journal of Environmental Engineering and Science **17**(2): 99-107.

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

Wagner, B., G. Daigger and N. Love (2022). "Assessing membrane aerated biofilm reactor configurations in mainstream anammox applications." Water Science and Technology **85**(3): 943-960.

Wang, H., G. Xu, Z. Qiu, Y. Zhou and Y. Liu (2019). "NOB suppression in pilot-scale mainstream nitritation-denitritation system coupled with MBR for municipal wastewater treatment." Chemosphere **216**: 633-639.

Wang, J., J. Liang, L. Sun, J. Shen and M. Wang (2021). "Achieving reliable partial nitrification and anammox process using polyvinyl alcohol gel beads to treat low-strength ammonia wastewater." Bioresource technology **324**: 124669.

Wang, R., A. Terada, S. Lackner, B. F. Smets, M. Henze, S. Xia and J. Zhao (2009). "Nitritation performance and biofilm development of co-and counter-diffusion biofilm reactors: modeling and

Chapter 2 – Literature Review: Overview of Biological Wastewater Treatment

2.1. Conventional wastewater treatment and nutrients removal processes

Conventional activated sludge (CAS) process is the general standard for biological pollutants removal from wastewater. In this system, aerobic microbes degrade the organic matter pollutants in wastewater to produce carbon dioxide and sludge. But following concerns over excessive nutrients in receiving water bodies, and the need to reduce nutrients in wastewater prior to discharge, incorporating biological nutrient removal through nitrification/denitrification and enhanced biological phosphorus removal became necessary. CAS also emerged as one of the preferred options for biological nutrient removal (BNR) from wastewaters because the system is effective, robust, simple to operate and reliable (Zhang and Liu 2022). BNR involves ammonium nitrogen (NH_4^+-N) conversion to nitrogen gas through (nitrification-denitrification) and phosphorus removal by polyphosphate-accumulating organisms (PAOs) through accumulation in specialized biomass (Hu, Houweling and Dold 2012). To be adaptable for nutrient removal, CAS is equipped with three zones; aerobic/anoxic/anaerobic. The aerobic zone is the fundamental component of the BNR systems, enhanced nitrogen removal happens in the anoxic zone, while the anaerobic zone is essential for the proliferation and enrichment of PAOs for phosphorus removal (Grady Jr, Daigger et al. 2011).

The alternating redox condition obtainable within the CAS system enhances the growth of multifunctional microbial community in CAS. However, nitrification is energy-intensive and expensive, sometimes requiring the addition of chemicals such as sodium bicarbonate to maintain desired alkalinity levels for nitrification. Denitrification may also require additional carbon, often beyond what is available in the influent in order to achieve acceptable nutrient discharge limits,

leading to increased costs (Zheng, Zhang et al. 2018). Both processes result in high sludge production which requires subsequent processing prior to either use or disposal (Wang, Xu et al. 2019). Therefore, the current CAS process for pollutant removal is unsustainable both in terms of energy efficiency, operational costs and carbon footprint (Verstraete and Vlaeminck 2011, Zhang and Liu 2022). As the challenge of maintaining sustainable wastewater treatment process intensifies, interest in sustainable technologies has also increased, driven by several factors including; eutrophication, stricter wastewater discharge regulations, aging infrastructures, operational expenditure (OPEX) reduction amongst others, thus facilitating the emergence of new and sustainable biological WWT processes like the membrane aerated biofilm reactor (MABR).

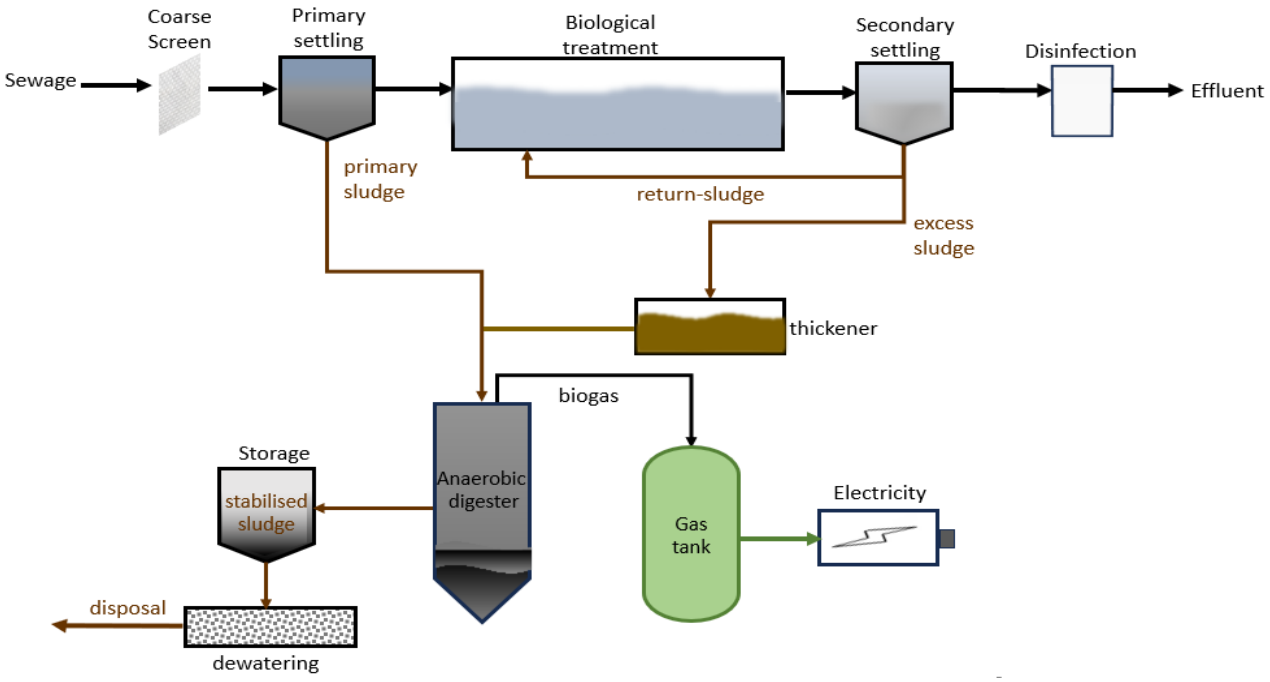


Figure 2.1. Schematic of classical CAS process with biological nutrient removal, disinfection, generation and sludge digestion, energy use and generation.

2.2. Overview of MABR application in biological wastewater treatment

The MABR technology was intensively studied for over three decades before its commercialization in 2015 (Silveira, Cadee and Bagg 2022). For municipal wastewater treatment, three commercial MABR products are currently available; OxyMem, ZeeLung and Fluence MABR by OxyMem Limited, Suez Water Technologies & Solutions, and Fluence Corporation respectively and commercial interest continues to grow for other types of wastewater (Guglielmi, Coutts et al. 2020, Uri-Carreño, Nielsen et al. 2021). Experimental and model results confirmed MABR has higher pollutant removal and energy efficiencies, compared to traditional wastewater treatment systems (Liao and Liss 2007, Peeters, Long et al. 2017, Corsino and Torregrossa 2022). Energy savings ranging from 15-86 %, have been reported for air and pure oxygen-based MABR systems (Casey, Glennon and Hamer 1999, Aybar, Pizarro et al. 2014, Syron, Semmens and Casey 2015). MABR design allows for single-stage simultaneous nitrification-denitrification (SND) within the same biofilm resulting in space-efficiency and reduced footprint (Sunner, Long et al. 2018). MABR modeling is also improving to facilitate better process design and operation (Carlson, He et al. 2021).

MABR technology breakthrough followed the development of a bubbleless hollow fiber membrane aerator capable of high oxygen transfer efficiency by Ahmed & Semmens, (1992). Thereafter, Pankhania et al. (1994) tested the feasibility of immobilising microorganisms on the hollow fibres aerator and successfully applied it to the treatment of synthetic municipal wastewater (Ahmed and Semmens 1992, Pankhania, Stephenson and Semmens 1994). Five years later, Semmens & Hanus, (1999) also using used synthetic municipal wastewater investigated the potential of single-stage simultaneous nitrification and denitrification (SND) using MABR, and reported >70 % BOD and nitrogen removal efficiencies. Effluent total suspended solids (TSS) for

this study was <30 mg/l (Semmens and Hanus 1999). These preliminary investigations catalysed myriad other studies covering reactor configuration (Shanahan and Semmens 2006, Liu, Yang et al. 2007), process parameters analysis (Hibiya, Terada et al. 2003, Cole, Semmens and LaPara 2004, Liu, Yang et al. 2007, Downing and Nerenberg 2008, Cao, Zhao et al. 2009, Li, Zhu et al. 2010, Shanahan and Semmens 2015, Li, Du et al. 2018), microbial community dynamics (Cole, Semmens and LaPara 2004, Liu, Tan et al. 2014, Tian, Zhao et al. 2015, Li, Du et al. 2018), membrane properties and biofilm characterization (Terada, Hibiya et al. 2003, Lin, Zhang et al. 2015, Tian, Zhang et al. 2015, Castrillo, Díez-Montero et al. 2019, Wu, Wu et al. 2019), MABR modeling (Park, Bae and Rittmann 2010, Shanahan and Semmens 2015, Ma, Domingo-Felez et al. 2017, Pérez-Calleja, Clements and Nerenberg 2022), and economic analysis (Aybar, Pizarro et al. 2014, Aybar, Pizarro et al. 2015). Several studies (Table 2.1) have reported effective application of MABR technology for other wastewater types, including; surface water (Li and Zhang 2018), industrial wastewater (Terada, Hibiya et al. 2003, Heffernan, Shrivastava et al. 2017), degradation of xenobiotic pollutant (Ohandja and Stuckey 2007, Syron and Casey 2008), pharmaceutical wastewater (Tian, Zhang et al. 2015), landfill leachates (Syron, Semmens and Casey 2015), and greywater treatment (Zhou, Li et al. 2020).

Additional advantages of the MABR technology includes; reduced operational tank volumes, high oxygen transfer rate and transfer efficiency (OTR and OTE), elimination of carbon adjustment and pH correctors amongst others (Gong, Yang et al. 2007, Dong, Wang et al. 2009, Martin, Boltz and Nerenberg 2012, Aybar, Pizarro et al. 2015, Lema and Martinez 2017).

MABR technology has also been found suitable for retrofitting and upgrading the capacities of existing plants (Kunetz, Oskouie et al. 2016, Houweling, Peeters et al. 2017, Peeters, Long et al. 2017, Underwood, McMains et al. 2018). However, the ability of the MABR to achieve

the same effluent quality as CAS at lower energy requirement makes it desirable for municipal wastewater treatment.

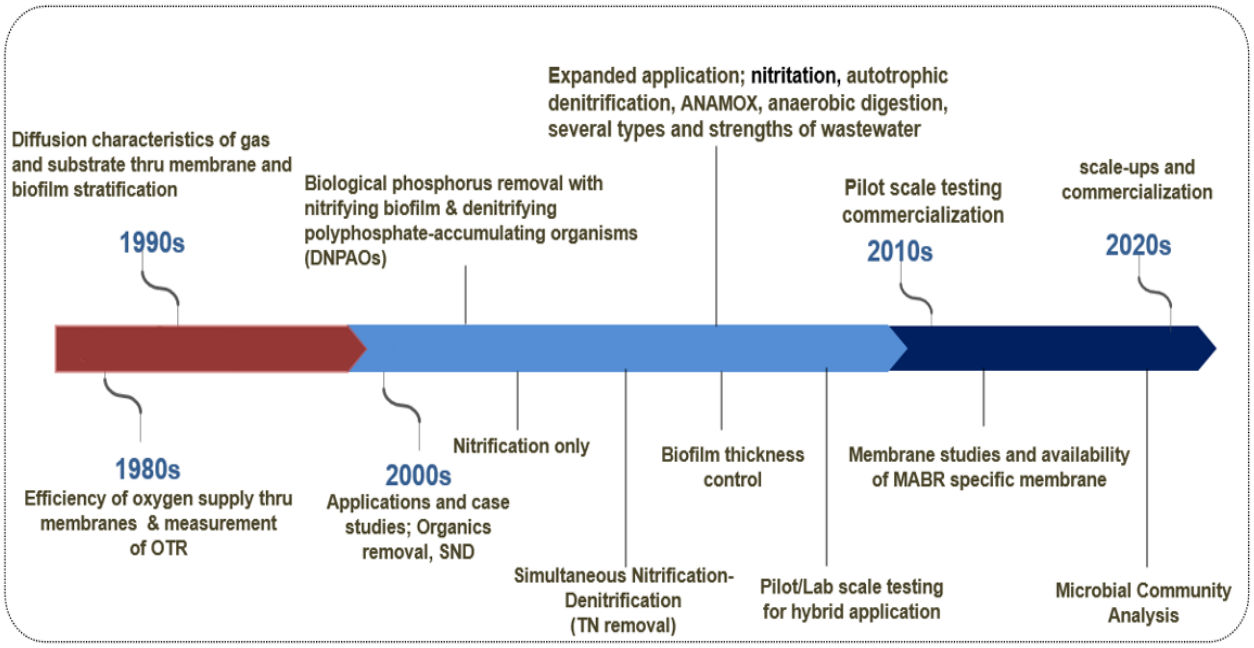


Figure 2.2. MABR development evolution over the years, from rudimentary investigations to commercialization.

2.3. Principle of the MABR technology

MABR is a 3-interface biofilm-based technology for pollutants removal from wastewater with high energy efficiency (Hu, Yang et al. 2009, Martin and Nerenberg 2012, Ukaigwe, Zhou et al. 2021, Li, Li et al. 2022). In MABR system, semi-permeable membrane immersed in wastewater is used to supply dissolved gas directly to biofilms growing on the membrane surface, while substrates from wastewater diffuse into the biofilms from opposite direction, thus electron donors and acceptors infiltrate the biofilm from opposing sides, producing a counter-diffusion effect. This effect produces maximum biological activity at the in the center of the biofilm, where both gaseous and liquid substrates are sufficient and ensures stability against shock loads and toxic inhibitors

(Nerenberg 2016). Oxygen transfer through diffusion eliminates oxygen wastage, producing up to 100% OTE, leading to improved organic carbon oxidation and nitrification rates (Hou, Jassby et al. 2019, Mehrabi, Houweling and Dagnew 2020, Nathan, Shefer et al. 2020). Additionally, nitrification performance in MABR is less susceptible to organic carbon limitation due to MABR design that allow nitrifying bacteria inhabit the interior of the biofilm (Houweling and Daigger 2019).

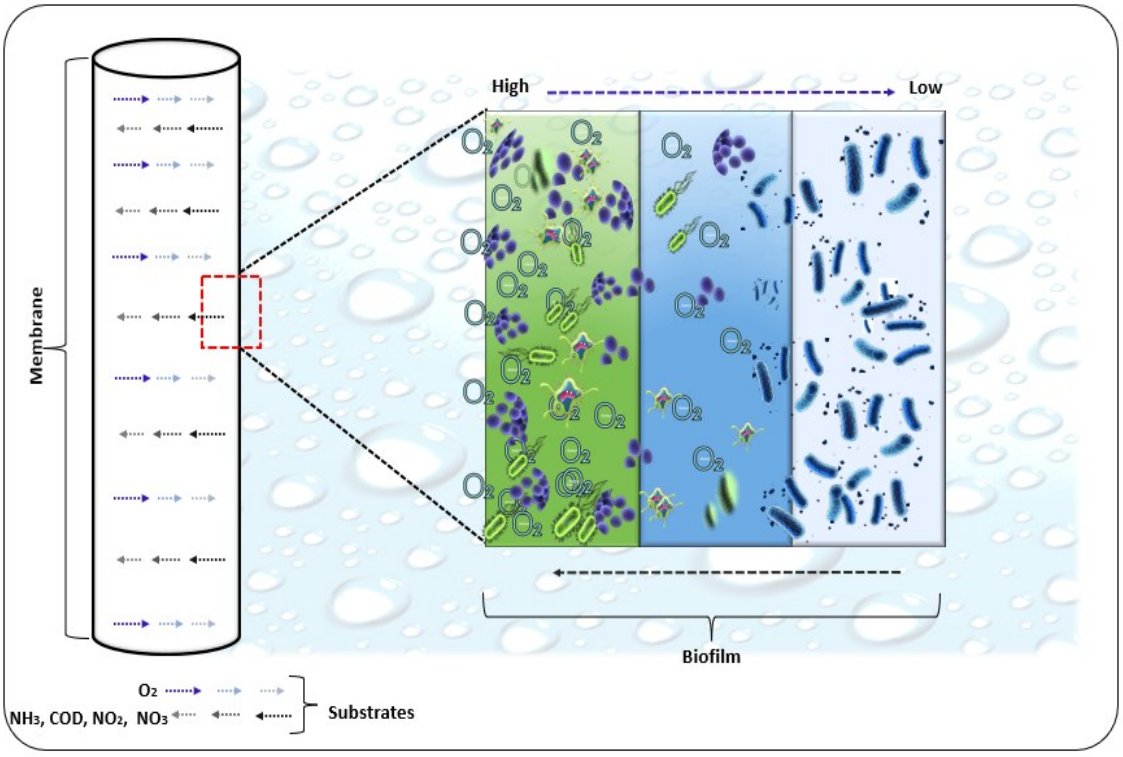


Figure 2.3. Conceptualized view of the 3-Phase MABR system made up of the air supply (gas), bulk liquid (water) and biofilm (solid).

Table 2.1. Summary of Selected MABR-Based Research from 1999 – 2023

Author	Title	Research Purpose	Influent Characteristics	Reactor Design & Process Parameters	Process Capability & Removal Efficiency
Semmens, M & Hanus, D (1999)	Studies of a membrane aerated bioreactor for wastewater treatment	Collect engineering and preference performance information on the behaviour of a membrane supported biofilm	Synthetic waste	Hollow-fiber membranes submerged in wastewater HRT (h): 6	COD removal, nitrification, denitrification and simultaneous sludge digestion 70 -75 %
Liao, B. Q. Liss, S. N. (2007)	A comparative study Between Thermophilic and Mesophilic membrane aerated biofilm reactors		Warm high strength synthetic wastewater	HRT (h): 24 pH: 6.8-6.9 Press (Psi): 4-6 Temp (^oC): 55; 18-25	COD removal 90 %
Liu et al, (2007)	Carbon membrane-aerated biofilm reactor for synthetic wastewater treatment	Demonstrate the effectiveness of carbon membrane as gas-permeable membrane in MABR for wastewater treatment.	Synthetic waste water	coal-based tubular carbon membrane (DLUT, Dalian) HRT (h): 8-16 pH: 7.5-8.0 Press (Psi): 2.3-2.9 Temp (^oC): 30 $\pm$ 2	COD removal and Nitrification 90 $\pm$ 2 % and 90 $\pm$ 4 %;
Ohandja, D. & Stuckey, D.C (2007)	Biodegradation of PCE in a Hybrid Membrane Aerated Biofilm Reactor	Demonstrate the effectiveness of MABR in PCE degradation	Synthetic waste water containing PCE	Flat sheet hybrid membrane aerated biofilm reactor HRT (h): 48-9 pH: Press. (Psi): Temp (^oC):	COD removal and sludge reduction 85-90%
Gong et al (2007).	Feasibility of MABR to achieve single-stage autotrophic nitrogen removal based on Anammox	Demonstrate the ability of using MABR reactor for CANON -type process	Synthetic waste water	Carbon tube walled-reactor HRT (h): 6 pH: 7.9 Press. (Psi): 5.8 Temp (^oC): 35	Nitrification 77%

Hu et al, (2009)	The development of a novel hybrid aerating membrane-anaerobic baffled reactor for simultaneous nitrogen and COD removal from wastewater	Achieve simultaneous removal of BOD and nitrification	Synthetic waste water	Hybrid: MABR installed into Anaerobic Baffled Reactor (ABR) HRT (h): pH: 7.5 ± 0.2 Press. (Psi): Temp (°C): 28 DO (mg/L):	COD removal and nitrification 96.8 and 83.5%
Cao et al, (2009)	Membrane-Aerated Biofilm Reactor Behaviors for the Treatment of High-Strength Ammonium Industrial Wastewater	Investigate the effect of reaction time, temperature, PH, C/N ratio and sludge concentration on COD removal rates and nitrification	Synthetic waste water and sludge liquor from sludge dewatering of municipal sewage treatment plant	MABR HRT (h): 8-5 pH: 7.5 Press. (Psi): Temp (°C): 25 - 30	
Dong et al, (2009)	Effect of DO on simultaneous removal of carbon and nitrogen by a membrane aeration/filtration combined bioreactor		Synthetic municipal waste water	Membrane aeration/filtration combined bioreactor (CMBR) HRT (h): pH: Press. (Psi): 25 Temp (°C): 28 -30	COD removal and nitrification 94.5% and 78.4 - 96.0%
Wei et al, (2012)	COD and nitrogen removal in facilitated transfer membrane-aerated biofilm reactor (FT-MABR)	Explore the effects of feed flow velocity on COD, NH4- N and TN	Synthetic waste water	Hollow fiber membrane-aerated biofilm reactor HRT (h): 12 pH: 7.8-8.4 Press. (Psi): 14 Temp (°C): 20 ± 2	COD removal 60.9 – 80 %
Lin et al (2015)	Nitrogen removal performances of a polyvinylidene fluoride membrane-aerated biofilm reactor		Artificial waste water	MABR with polyvinylidene fluoride (PVDF) hollow fiber membranes HRT (h): 24 pH: 7-8 Press. (Psi): 7.25 Temp (°C): 20 ± 2	Simultaneous nitrification and denitrification >75%

Yi and Zhang (2017)	Pilot scale treatment of polluted surface waters using membrane-aerated biofilm reactor (MABR)	Effect of process parameters on nitrogen removal efficiencies	Artificially simulated polluted surface water	Pilot scale MABR in continuous mode HRT (h): 12-48 pH: Press. (Psi): 1.45 - 2.2 Temp (°C):	Nitrogen removal through simultaneous nitrification and denitrification 60.3 - 92.2%
Narinder et al., (2018)	MABR as a low-energy compact solution for nutrient removal upgrades – results from a demonstration in the UK	Demonstrate the treatment efficacy of MABR technology under UK conditions	Sewage	Reactor operated in a similar way to a CAS system HRT (h): 4.3-3.8 pH: Press. (Psi): Temp (°C): 10.3 -18.3 DO (mg/L): 2-3	NH4+-N and BOD_5 in the effluent within their target limits. 90 and 96%
Wu et al., 2019	Comparison study on the performance of two different gas-permeable membranes used in a membrane-aerated biofilm reactor	Investigate the effects of membrane type on MABR performance.	Synthetic low-strength wastewater	Tempt. (°C): 20 ± 0.5 Pres. pH: DO: HRT (h):12; 20	PVDF membrane was more favorable for the attachment of microorganisms than the PP membrane due to the surface roughness.
Bunse et al., 2020	Membrane aerated biofilm reactors for mainstream partial nitritation/anammox: Experiences using real municipal wastewater	Investigated the potential of MABR to achieve mainstream nitrogen removal via partial nitration/anaerobic ammonium oxidation (anammox)	Real municipal wastewater	Tempt. Pres. pH: DO: HRT (h):28.8 - 7.2	Mainstream PN/A is possible with MABR NH_4^+-N, TN (70–90, 60–80%)
Ma et al., 2022	Intermittent aeration to regulate microbial activities in membrane-aerated biofilm reactors: Energy-efficient nitrogen removal and low nitrous oxide emission	MABRs operated under continuous versus intermittent aeration strategies to study the impact on long-term N conversions.	Synthetic municipal wastewater	Tempt. (°C): 20 -30 Pres. (bar): 0.05 – 0.4 pH: DO (mg/L): 2.95 ± 0.83 – 3.14 ± 0.91 HRT	Mainstream PN/A, with high NH_4^+ and total nitrogen removal possible using MABR

Zhou et al., 2022	The influent COD/N ratio controlled the linear alkylbenzene sulfonate biodegradation and extracellular polymeric substances accumulation in an oxygen-based membrane biofilm reactor	Systematic investigation of EPS dynamics in an MABR in response to systematic changes in influent COD/TN ratio	Simulated grey water containing linear alkylbenzene sulfonate (LAS)	Tempt. (°C): 21.5 ± 0.53 Pres. (psi): 1 pH: 6.8 -7.1 DO (mg/L): 0.08 – 0.62 HRT (h):10	High quantity of EPS in biofilm improves greywater treatment. EPS accumulation is dependent on COD/TN ratio.
Ukaigwe et al., 2023	Structural and Microbial Dynamics Analyses of MABR Biofilms	Biofilm thickness management and reactor performance stability	Synthetic municipal wastewater	Tempt. (°C): 22 ± 0.3 Pres. (psi): 2 pH: 7.1 – 8.2 DO (mg/L): 0.2 – 0.6 HRT (h):48 -2.5	Intermittent membrane cleaning with water effectively controlled biofilm thickness

P.S.: Missing values not indicated in the paper

2.4. MABR design and operation considerations

Parameters critical to the design and operations of the MABR include: the membrane, lumen gas, and the biofilm. These parameters are reviewed below.

MABR membrane serves as biofilm attachment surface, ecosystem to microbes, gas supply channel and the technology cost indicator (Semmens, Dahm et al. 2003, Liu, Yang et al. 2007, Aybar, Pizarro et al. 2015). Additionally, membrane material also affects the type of microorganisms present in the biofilm and the effectiveness of their actions (Nisola, Orata-Flor et al. 2013). This multifunctionality makes them integral to the success of the MABR technology. A balance is usually required between membrane properties and membrane cost (Casey, Glennon and Hamer 1999, Semmens and Shanahan 2005, Hou, Li et al. 2013). The membrane material is expected have suitable characteristics to support biofilm growth and control wetting, the capacity to maintain high and efficient gas transfer rates to the biofilm, and the strength to withstand mechanical and chemical damages (Stricker, Lossing et al. 2011). Three categories of membrane materials; microporous, dense (nonporous) and composite have been extensively investigated and applied in MABR systems.

2.4.1. Membrane materials

Microporous membranes are made from organic polymers such as polyvinylidene fluoride (PVDF), polytetrafluoroethylene (PTFE) and polypropylene (PP) (Nerenberg 2016, Xiao, Zhou et al. 2021). They can be either hydrophilic or hydrophobic, have low-cost advantage, and can achieve bubble-free aeration at pressures below their bubble points (Lu, Shen et al. 2022). Gas transfer into biofilm using microporous membrane is through the membrane pores in contrast to gas transfer via diffusion in dense membrane. This makes the membrane pores vulnerable to bacteria colonization and particle deposition on the pore walls, which may result in pore blocking, wetting and decreased oxygen transfer rates (OTRs) (Rothemund, Camper and Wilderer 1994). Consequently,

microporous membranes application have mostly been limited to lab scale investigations (He, Wagner et al. 2021).

In contrast, scale-up and commercial MABR installations have mostly been made from dense membranes. Gas transfer in dense membrane is via diffusion which eliminates bubbles formation, thus allowing high pressure operations. This process naturally increases the concentration gradient, and therefore, the mass transfer rate (Ahmed and Semmens 1992). Dense membrane also have increased strength, and durability (Lu, Bai et al. 2021), but high resistance to mass transfer is common due to thick membrane walls. However, with recent technological development, dense membranes with thinner walls are now available. For instance, OxyMem silicone polydimethylsiloxane (PDMS) is a dense membrane with outer diameter of 510 μm and wall thickness of 100 μm, and has been successfully applied in OxyMem operations. However, dense membranes are generally more expense than microporous membranes. Examples of dense membrane that have been applied in MABR operations include polymethyl pentene (PMP) and silicone polydimethylsiloxane (PDMS).

An alternative to microporous and dense membranes is the composite membrane. They are made by coating or embedding a thin and dense polymer layer into a microporous membrane and thus have the advantages of microporous and dense membranes, and some of the disadvantages like clogging as well. Additionally, high cost is a known disadvantage. Typical coating material for composite membranes is *L*-3,3-dihydroxyphenylalanine (*L*-DOPA). Nisola et al., (2013) reported hydrophobic composite membranes are more suitable for bacterial adhesion than their hydrophilic counter part (Nisola, Orata-Flor et al. 2013). Although other investigators have suggested that hydrophilic membrane surfaces better promote biofilm adhesion (Hou, Li et al. 2013, Wu, Wu et al. 2019).

Many factors control gas transport through porous microporous membranes including porosity, overall thickness and pore size (De Meis 2017). Transport mechanism is defined by pore sizes. For instance, at larger pore sizes (0.1 - 10 µm) conventional flow exist, when pore size < 0.1µm, Knudsen flow occurs, however, in between these pore sizes the flow is a combination of flow types. Pore sizes of microporous membranes that have been tested in MABR have ranged from small at 0.02 - 0.05µm to large at 0.1 - 0.3µm (Ahmed and Semmens 1992).

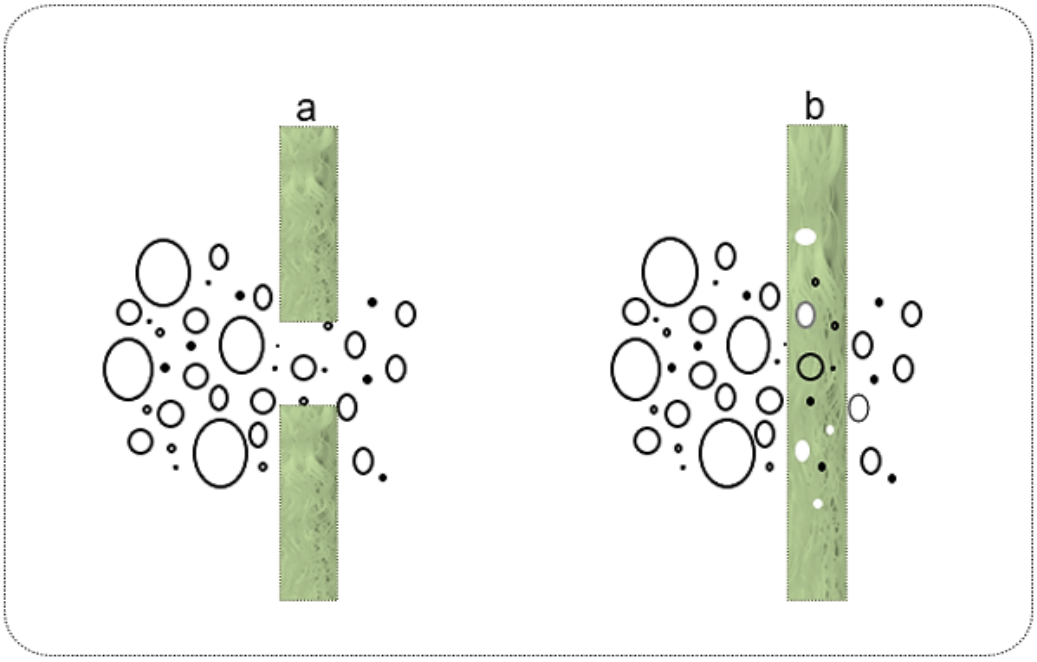


Figure 2.4. Gas transport mechanism through porous and nonporous membranes a) pore flow (porous membrane). Pressure difference between the pore entrance and exit drives the flow b) solution – diffusion (dense membrane). Gases dissolve into the membrane polymer matrix and diffuse through the thickness of the membrane.

Table 2.2. Summary of MABR Membrane Properties

Membrane	Advantages	Limitations
Microporous	High specific surface area Low cost Support the fastest rates of gas transfer	limited to low intramembrane pressure operations due to vulnerability to bubbling. Most susceptible to clogging and wetting as pores become hydrophilic via a conditioning film Wetting causes severe reduction in oxygen transfer rate
Dense	Support high intra-membrane pressure and thick biofilm Reduces oxygen mass transfer resistance Prevents lumen wetting	Increased wall thickness Thickness, >100 μm in comparison to <10 μm for microporous membranes. Introduces significant diffusional resistance that can slow the rate of gas transfer
Composite	Promote fast gas transfer has high bubble point	Significantly more expensive than conventional membranes Cost maybe prohibitive for upscale MABR operations

2.4.2. Membrane specific surface area

High membrane specific surface area is one of the major advantages of MABR technology, as it allows for high density of pollutant-reducing microbes in the reactor, hence, low hydraulic retention time can be applied. Thereby, minimizing capital costs and process footprint (Nerenberg 2005, Syron and Heffernan 2017). Hollow fiber membranes used in MABR operations can be made with outside diameters as little as 75 μm, providing specific surface area (membrane surface area / reactor volume) as high as 3500 m^2/m^3 (Martin and Nerenberg 2012). Membrane modules with specific surface areas ranging from 45 to 5107 m^2/m^3 have been applied in MABR process operations, and results demonstrate the dependence of pollutant removal rates on membrane specific surface area (Pankhania, Stephenson and Semmens 1994, Terada, Hibiya et al. 2003, Terada, Yamamoto et al. 2006, Syron and Casey 2008, Li and Zhang 2018, Li, Li et al. 2022, Li, Bao et al. 2023).

2.5. MABR lumen gas

Depending on the treatment objective, different gases can be utilized in MABR operations. Typically, pressurized air is used as the lumen gas, although pure oxygen (O_2) or oxygen-enriched air can also be used when treating high-strength or industrial wastewaters. Hydrogen (H_2) and methane (CH_4) have also been applied as supply gases in MABR operations (Lai, Zhong et al. 2016, Zhou, Ontiveros-Valencia et al. 2019). In these types of system, oxygen serves as an electron acceptor for aerobic degradation, while either hydrogen or methane will serve as the electron donor for autotrophic denitrification (Xia, Zhong et al. 2010, Nerenberg 2016).

MABRs are particularly relevant as safer route for the delivery of hydrogen to pollutants because they deliver the gas without bubble formation, which is a limitation of the traditional denitrification process (Nerenberg 2016, Lema and Martinez 2017). Hydrogen also supports autotrophic bacteria, thereby eliminating the need for an organic carbon. Furthermore, hydrogen is non-toxic, cost-effective, yields low biomass and produces high quality effluent (Tang et al., 2011). H_2-based MABR has been successfully applied in the removal of oxyanions from municipal wastewater and drinking water (Sun, Wang et al. 2015). Recently, Pang et al., (2023) effectively used H_2-based MABR for efficient and simultaneous removal of NO_3^--N (95.4 %) and NH_4^+-N (99.5 %) from synthetic wastewater. Scale-up and commercial development of the H_2-based MABR began in 2005 by APTwater, LLC.

O_2-based MABR is currently the most studied and most applied approach in MABR operations. Several studies on MABR have been carried out focusing on the potentials of the O_2 – MABR to improve organic carbon degradation (Perez-Calleja, Aybar et al. 2017), nutrients removal via simultaneous nitrification-denitrification (SND) (Semmens, Dahm et al. 2003, Lin, Zhang et al. 2015, Peeters, Long et al. 2017), partial-nitrification (Terada, Yamamoto et al. 2006, Nisola, Orata-Flor et al. 2013, Mehrabi, Houweling and Dagnew 2020), partial nitrification-anammox (Bunse,

Orschler et al. 2020, Chen, Cao et al. 2022, Li, Li et al. 2022), reducing energy requirements (Côté, Peeters et al. 2015, Lema and Martinez 2017) and greenhouse gases emission (Kinh, Riya et al. 2017, Ma, Piscedda et al. 2022). Commercially available MABR are all O_2-based (Heffernan, Shrivastava et al. 2017, Houweling, Peeters et al. 2017, Houweling and Daigger 2019, Guglielmi, Coutts et al. 2020).

2.6. Biofilms

Biofilms are natural, heterogeneous, multiphase materials consisting primarily of microbial cells, that develop on all kinds of surfaces. They live in organized systems embedded in self-produced extracellular polymeric substances (EPS), representing a protected mode of existence which allows cells to survive over a wide range of different and sometimes extreme environmental conditions (Denkhaus, Meisen et al. 2007, Hu, Xu et al. 2013, Boltz, Smets et al. 2017). Biofilm heterogeneity and complex functionalities offer research opportunities in several fields; indeed, they have been integral to wastewater treatment for over 100 years, due to their ability to accumulate high biomass densities, long biomass retention times and internal zonal stratification (Sehar and Naz 2016).

Biofilm formation is a complex process that occur in consecutive series; a) conditioning film (initial attachment), b) microcolony formation (transport of microorganism and other particles to the surface), c) microbial attachment and growth process (maturation), d) movement of substrates through the bulk liquid and biofilm matrix into the biofilm core, e) reaction at the active sites, f) mass transfer from products from biological reactions, and g) biofilm detachment through erosion, abrasion and sloughing (Melo 2003, Carniello, Peterson et al. 2018). Biofilm is the foundation of the MABR technology, its robust qualities are well exploited in MABR processes.

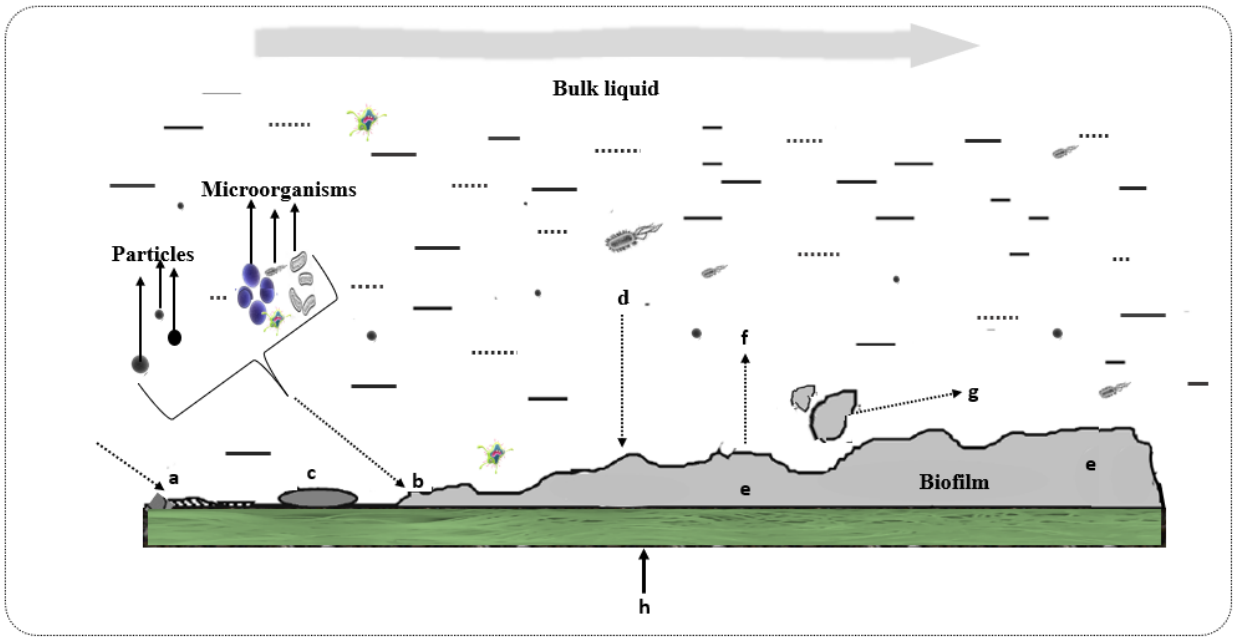


Figure 2.5. The process of biofilm development occurring in consecutive steps from a) initial attachments, b) movement of microorganism and other particles to the membrane surface (h), c) growth process, d) substrate transport into biofilm, e) biological reaction at active sites, f) movement of reactions products, and finally to g) biofilm detachment.

2.6.1. MABR unique biofilm

The MABR biofilm is most important feature of the MABR system, and is responsible for metabolizing pollutants in wastewater. These biofilms are referred to as counter-diffusional biofilms in contrast to co-current biofilms from conventional biofilm systems (Nerenberg 2016). Studies have shown that MABR counter-diffusional biofilms has higher pollutant removal capacity than conventional biofilm due to its unique mass transfer process that position microbial activities at the biofilm core, in contrast to surface activity in conventional biofilms. Additionally, in conventional biofilms, nitrifiers grow on the surface and are exposed to the wastewater, where they can be easily sloughed off, whereas, in MABR, nitrifiers are protected from washouts in MABR biofilm. Moreover, counter-diffusional biofilm enables the formation of microenvironments with multiple regions (aerobic, anoxic and anaerobic) that enable the growth of specific bacteria (*e.g.* nitrifiers and anaerobic nitrifiers) to support simultaneous nutrient and organic carbon removal processes

within the biofilm. (Nerenberg 2016, Zhou, Li et al. 2020, Ukaigwe, Zhou et al. 2021). Oxygen limitation abound in conventional biofilms, while oxygen limitation does not occur in biofilm, although ammonia and COD may become limiting (Syron and Casey 2008). These unique characteristics promote higher output in all wastewater treatment performance indices including; effluent quality, percentage pollutant removal, surface loading and pollutant removal flux.

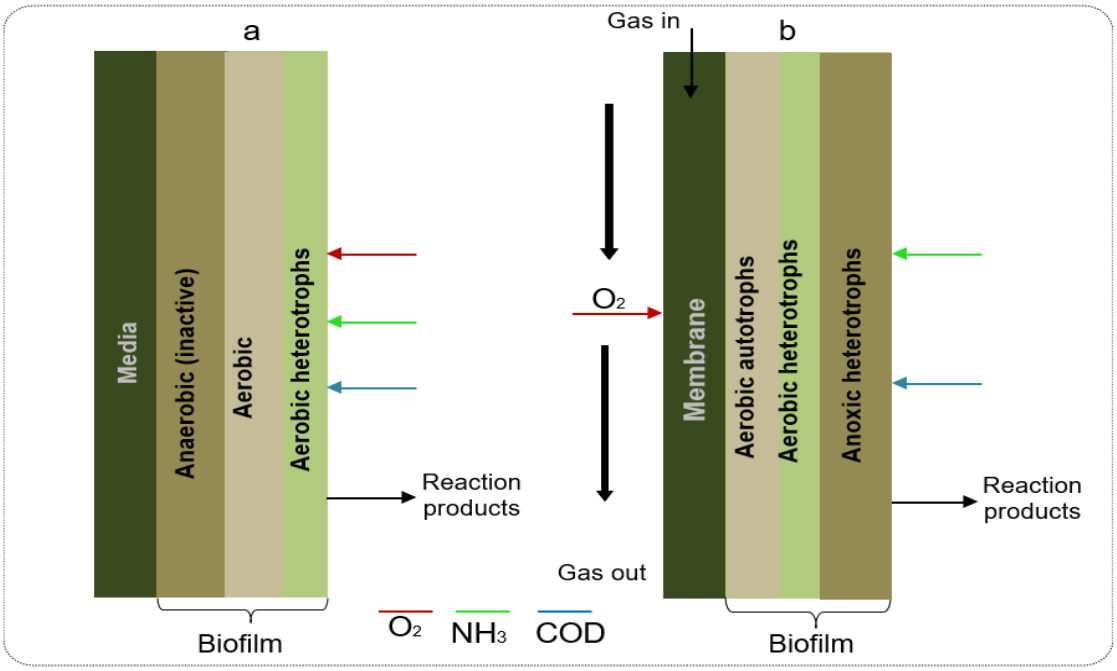


Figure 2.6. Schematic of conventional and MABR biofilms, Showing fundamentally different stratifications and the movement pattern of oxygen and substrates into the biofilms. a) conventional biofilm b) MABR biofilm.

2.6.2. Biofilm thickness

The main challenge of biofilm-based processes is the uncontrolled growth of microorganisms which results in increased biofilm thickness and process instability (Janczewski and Trusek-Holownia 2016, He, Wagner et al. 2021). However, maintaining a healthy biofilm with appropriate thickness to efficiently remove pollutants from wastewater remains the goal of biofilm-based wastewater treatment processes. Biofilm thickness influences several components of the

biofilm structure and even control both the functionality and stratification of microbial community structure in a biofilm (Nerenberg 2016, Suarez, Piculell et al. 2019, Sanchez-Huerta, Fortunato et al. 2022). Desirable biofilm thickness however depends on treatment objective and wastewater type.

Thinner biofilms enhance oxygen and substrate mass transfer though may result in low fluxes when they are insufficient, while thicker biofilm increases diffusive resistance, but allow for the development of stratified layers to achieve simultaneous organic carbon and nitrogen removal (Rittmann and McCarty 1980, Martin and Nerenberg 2012). MABR process, however, is able to overcome diffusional limitation when it supplies oxygen to biofilm at elevated pressures (Syron and Casey 2008, Chen, Cao et al. 2022). Excessive biofilm thickness on the other hand increases flow short circuiting, high liquid head loss and limits process performance (Pankhania, Stephenson and Semmens 1994, Semmens, Dahm et al. 2003, Elsayed, Hurdle and Kim 2021). Although, Elsayed et al., (2021) recently reported that substrate transport within biofilms was too fast to be hindered by biofilm thickness.

Literature shows that mass transfer characteristics of MABR biofilm with thickness of 0 - >4000 μm has been studied (Casey, Glennon and Hamer 2000, Semmens 2005, Heffernan, Shrivastava et al. 2017, Bunse, Orschler et al. 2020, Taşkan, Hasar and Lee 2020).

2.6.3. Biofilm thickness management

Biofilm thickness is the single most critical MABR performance parameter and has been the most extensively studied. But despite many years of study, the fundamental understanding of the factors controlling biofilm properties within the MABR is still limited. Several efforts to control biofilm excessive growth have been reported in literature including; back-washing; consisting of high-shear air scouring, followed by flushing with clean water (Pankhania, Stephenson and Semmens 1994, Terada, Hibiya et al. 2003, Pellicer-Nàcher, Franck et al. 2014), air-bubbles induced sloughing (Semmens 2005, Syron, Semmens and Casey 2015), continuous cleaning (Suzuki, Hatano

et al. 2000) and intermittent scouring at high frequency and low shear (Stricker, Lossing et al. 2011). Stricker *et al.*, (2011) also suggested very high biofilm specific surface area could contribute to biofilm control by keeping surface loading rate low. Pellicer-Nàcher *et al.,* (2014) recommended scouring because the outer anoxic biofilm layer in a stratified MABR had lower strength in comparison to the inner nitrifying layer.

Flow velocity has also been reported to influence mass transfer in the diffusion boundary layer, the biomass detachment rate from the biofilm, and the maximum biofilm thickness attained (Casey, Glennon and Hamer 2000, Da Silva, Matsumoto et al. 2018). Da silva et al., (2018) used two recirculation flow velocities of 0.025 and 0.065 m/s to study biofilm thickness control in an MABR operated in sequential batches and concluded that recirculation velocity is a promising option for biofilm thickness control in MABR (Da Silva, Matsumoto et al. 2018). However, the most commonly reported method is intermittent high shear combined with air scouring, as high shear forces can remove fractions of detachable biofilm resulting in thin compact biofilm layers (Martin, Boltz and Nerenberg 2012, Paul, Ochoa et al. 2012). Effective control of biofilm thickness is essential to maintain optimum thickness and remains the key challenge in MABR technology advancement (Nerenberg 2005).

2.7. Operation strategy

The operation of MABR systems for municipal wastewater treatment is defined by three elements; gas supply mechanism, membrane orientation and membrane packing density.

2.7.1. Gas supply mechanism

Gas is supplied to the MABR process through either flow-through or dead-end supply modes (Semmens and Shanahan 2005, Martin, Boltz and Nerenberg 2012, Lema and Martinez 2017). In the flow-through mode, the gas is continuously pumped through the hollow fibers, and vented to keep the partial pressure of oxygen high along the membrane. Thus, oxygen transfer rates (OTR) are high, but oxygen transfer efficiency (OTE) is low. While in the dead-end mode, one end of the fibers is sealed and the membrane pressurized with the gas, a process with the capacity to produce 100% OTE (Ahmed and Semmens 1992, Perez-Calleja, Aybar et al. 2017). Dead-end modes are however susceptible to back-diffusion of inert gases, which can reduce average oxygen transfer rates (OTR), consequently decreasing the average contaminant removal fluxes. Perez-Calleja *et al.,* (2017) OTR and OTE are key parameters for determining MABR performance (Côté, Peeters et al. 2015, Houweling, Peeters et al. 2017).

Many researchers have studied the influence of different operating parameters including microbial community on the performance of MABR treating municipal wastewater using either the flow-through or dead-end gas supply modes (Cote, Bersillon et al. 1988, Ahmed and Semmens 1992, Cole, Shanahan et al. 2002, Perez-Calleja, Aybar et al. 2017, Wu, Wu et al. 2019). Côté, *et al* (1988) had suggested that flow-through system should be the preferred operation mode as dead-end operation decreases performance. However, increase in gas flow rates (Li, Zhu et al. 2010) and intermittent degassing (Castagna, Zanella et al. 2015) have been suggested as techniques for improving the dead-end operation. Perez-Calleja *et al.,* (2017) aligns with the theory of intermittent degassing and suggested periodic venting as the strategy that combines the advantages of flow-through and dead-end modes, thereby maximizing both the OTR and OTE.

2.7.2. Membrane orientation

When the MABR arrangement is such that influents run parallel to the membranes, a liquid diffusion layer (LDL) forms at the biofilm surface. It introduces diffusional resistance, which may benefit the system by slowing the loss of gas from the membrane to the bulk liquid. However, most commonly, it is desirable to minimize the LDL, as it decreases the flux of bulk liquid substrates into the biofilm. Perpendicular flow eliminates the LDL, though a greater drop in pressure results (Martin and Nerenberg, 2012).

2.7.3. Membrane packing density

By convention, MABR are designed with the hollow-fiber membranes in bundles and the bundles placed in rows (Castrillo, Díez-Montero et al. 2019, He, Wagner et al. 2021). In practice, the membranes are potted to create a module, and modules are installed into cassettes for deployment in bioreactors (He, Wagner et al. 2021). The bundle configuration and size determine the packing density, which invariably affects mass transfer performance between the bulk liquid and biofilm within the reactor (Castrillo, Díez-Montero et al. 2019). In the hybrid systems, packing density is a critical parameter for sizing the MABR zone (He, Wagner et al. 2021). Bundling of membranes often render the inner membranes useless, and significantly decrease the membrane specific surface area. While high packing densities 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, Jassby et al. 2019). High packing density also leads to clogging/dead zones (Wu and Chen 2000, Castrillo, Díez-Montero et al. 2019).

Membrane packing density as well as the placement of the membranes must be designed to avoid the formation of dead zones or preferential flow routes. More investigations are needed on means of increasing packing density, while avoiding clogs and dead zones.

2.8. MABR performance

2.8.1. Organic carbon removal

The potential for high specific oxidation rates especially for soluble carbonaceous pollutants oxidation was the initial motivation for study of the MABR technology (Ohandja and Stuckey 2007, Syron and Casey 2008, Dong, Wang et al. 2009). Ab initio, MABR had displayed the potential for higher oxygen transfer rates than conventional biofilm systems as very high organic carbon oxidation rates have been reported from the years of preliminary MABR studies till date. (Yeh and Jenkins 1978) reported 91% BOD from an MABR with a high loading rate of 4.9 $g_{BOD}\ m^{-2}\ day^{-1}$ and a detention time of 2 h. Dong et al, (2009), reported >90% COD removal from synthetic sewage. Ohandja and Stuckey, (2007) also demostrated the capability of MABR to remove organic pollutant and reported 90% COD removal from synthetic waste water containing PCE. More recently, Ukaigwe et al., (2021) reported 98% TCOD removal from real primary effluent (PE) using MABR at a high surface loading rate (SLR) of 20 $g_{BOD}\ m^{-2}\ day^{-1}$. Organics removal efficiency in the range of 50 - >90% has been reportedly achieved in the treatment of municipal wastewater using MABR (Sun, Li et al. 2020, Ukaigwe, Zhou et al. 2021).

2.8.2. Ammonia/nitrogen removal

One of the many advantages of the MABR technology is efficient ammonia and total nitrogen removal from various wastewater types. Three principal reasons have been advanced for this ability; unique microbial stratification profile in MABRs, the ability of MABRs to maintain a relatively high ratio of nitrifiers to heterotrophs and the protection of nitrifiers from bulk liquid inhibitors offered by a heterotrophic layer adjacent to the biofilm−liquid interface (Syron and Casey 2008). Generally, ammonia removal >70% have been reported for MABR (Hu, Yang et al. 2009, Sunner, Long et al. 2018, Bunse, Orschler et al. 2020), but ammonia removal as high as 100% have also been reportedly achieved using MABR (Terada, Yamamoto et al. 2006). Ammonia removal

efficiencies >80 % have also been reported for hybrid MABRs (Kunetz, Oskouie et al. 2016, Carlson, He et al. 2021). Terada et al., (2006) and Hu et al, (2009) both reported >80% total nitrogen removal efficiency using hybrid MABR.

2.8.3. Removal of phosphorus

Studies evaluating MABR potential for biological phosphorus removal are still relatively novel and limited, but growing in reputation. MABR process can enable the creation of alternate anaerobic/aerobic conditions within its biofilm for the domestication of phosphate accumulating organisms (PAOs) and subsequent phosphorus removal (Kunetz, Oskouie et al. 2016). However, preliminary results suggests that biological phosphorus removal is achievable in the MABR, when the system is operated in sequencing batch mode with a biofilm removal stage (Sun, Wang et al. 2015). MABR can also be operated in a hybrid mode as membrane aerated biofilm reactor/activated sludge (MABR/AS) system at reduced solids retention time (SRT) without compromising process performance to integrate phosphorus removal (Houweling, Peeters et al. 2017). Terada et al., (2006) created isolated regions conducive to the growth of nitrifying bacteria and denitrifying polyphosphate-accumulating organisms (DNPAOs) in a sequencing batch membrane biofilm reactor (SB/MABR) through bulk DO control to validate the simultaneous nitrogen and phosphorus removal capability of the reactor, and obtained high phosphorus removal efficiency of 90%. More recently, Sun et al., (2015), also used a hybrid SB/MABR reactor to study phosphorus removal from municipal wastewater with DO and other parameter control and obtained total phosphorus removal efficiency >85%. Overall, the application of MABR for biological P-removal, relies on the ability to maintain the correct DO concentration within the system in all the stages in addition to retaining the right Polyphosphate-accumulating organisms (PAOs) strain (Sun, Wang et al. 2015).

The potential of MABR for the treatment of wastewater with other type pollutants have also been more recently explored and high removal efficiencies for various pollutants reported (Syron, Semmens and Casey 2015, Nerenberg 2016, Zhou, Ontiveros-Valencia et al. 2019).

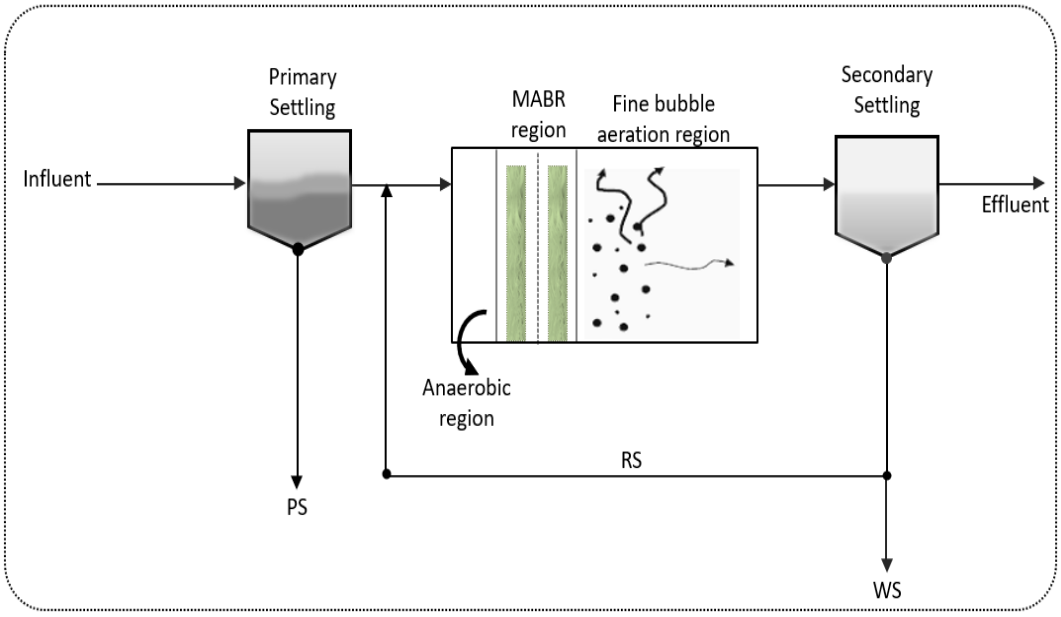


Figure 2.7. Schematic of a hybrid MABR process with an anaerobic region to enable biological phosphorous removal.

2.9. Nitrogen removal pathways

Currently, there are several nitrogen-removing pathways from wastewater that are thermodynamically feasible, but the most widely investigated and applied pathways include; "two-stage" nitrification and denitrification (ND), single-stage simultaneous nitrification-denitrification (SND), partial nitrification-denitrification (PND) (nitritation-denitritation), partial nitrification-annamox (PNA), complete ammonia oxidation (comammox), coupled aerobic-anoxic nitrous decomposition operation (CANDO) and uptake of ammonium, nitrate and nitrite by algal (Wang, Xu et al. 2019, Yang, Xu et al. 2019, Salbitani and Carfagna 2021, Zou, Zhou et al. 2022).

2.9.1. Complete nitrification and denitrification (ND)

The conventional pathway of biological nitrogen removal from wastewater is through the sequential and complimentary reactions of nitrification and denitrification commonly referred to as "two-stage" ND. During nitrification, ammonia is converted into nitrite or nitrate under aerobic conditions, while the nitrite or nitrate is converted into nitrogen gas under anoxic conditions during denitrification. "Two-stage" ND is the foremost and most studied (Liu, Kim et al. 2020) pathway, and has been successfully applied in the treatment of various wastewater types including; mainstream, high strength, and industrial waste water. In the "two-stage" ND, nitrification produces the electron acceptor (nitrite or nitrate) (eqn. 1 and 2) needed in denitrification, and increases the system pH. Denitrification generates the alkalinity required in nitrification, while nitrification reduces the pH, raised in denitrification. Complete denitrification consists of sequential reductive reactions from nitrate to nitrogen gas with organic carbon as the electron donor (eqn. 3). But due to high energy and carbon requirements associated with this pathway, simultaneous nitrification and denitrification (SND) was proposed. Nitrification is performed in two steps, nitritation and nitratation, accomplished by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), respectively.

2.9.2. Single-stage simultaneous nitrification-denitrification

The MABR technology principle is based on single-stage simultaneous nitrification-denitrification (Lin, Zhang et al. 2015, Li and Zhang 2018)**.** In the early days of MABR technology development, Timberlake et al., (1988) showed that the high oxygen concentrations at the MABR biofilm interface would support nitrification, an aerobic heterotrophic layer above this would facilitate COD pollutant removal, and an anoxic layer close to the biofilm−liquid interface would allow denitrification (Timberlake, Strand and Williamson 1988). Subsequent studies successfully demonstrated the capacity of the MABR to achieve SND (Syron and Casey 2008). Lin et al., (2015)

reported >75% nitrogen removal from synthetic wastewater through the SND pathway. Li and Zhang (2018) also reported >90% nitrogen removal from surface water through SND. Nutrient removal through SND is dependent on the ability biofilm to create a conducive environment for the coexistence of nitrifying and denitrifying bacteria, although, the exact mechanism is still not fully understood as a result of the broad diversity of the microbial community (Chai, Xiang et al. 2019), however, studies have confirmed that SND is more energy efficient than "two-stage" ND.

Nitrification

$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$ …… *(1)*

$NO_2^- + 0.5O_2 \rightarrow NO_3^-$ …… *(2)*

Denitrification

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ …… *(3)*

Complete nitrification

$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$ …… *(4)*

Partial nitrification

$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$ …… *(5)*

$NH_4^+ + 1.32\ NO_2^- \rightarrow 1.02N_2 + 0.26\ NO_3^- + 2H_2O$…… *(6)*

$NH_4^+ + 0.85O_2 \rightarrow 0.11NO_3^- + 0.445N_2 + 1.13H^+ + 1.43\ H_2O$ …… *(7)*

2.9.3. Partial nitrification-denitrification (PND)

Partial nitrification–denitrification (PND) also known as nitrite shunt or nitritation, is an alternate pathway for nitrogen removal. Here NH_4^+ is partially oxidized to NO_2^- , which is then reduced directly to nitrogen gas. This requires less oxygen and less organic carbon (1.5 mol O_2 and 3 mol organic carbon per 1 mol NH_4^+ to N_2) (eqn. 5) (Wagner, Daigger and Love 2022), making the process more energy efficient and more sustainable. This process is hinged on the fact that nitrite is the intermediate product of nitrification and denitrification processes, therefore the operation can be terminated at the nitritation stage, which translates to 25% less oxygen use. The key to the generation and sustenance of partial nitrification within a system is to provide NOB inhibiting conditions, without retarding the activities of AOBs and denitrifiers, so as to enable the accumulation of nitrite (Liu, Kim et al. 2020). The concept of PND is relatively new in MABR systems, but studies have shown that some of the techniques of NOB suppression in other biological systems like regulating free ammonia and free nitrous acid concentrations, control of SRT/HRT, high/low temperature conditions, low DO condition *etc* (Yang, Peng et al. 2007, Blackburne, Yuan and Keller 2008) can be successfully applied in MABR depending on wastewater conditions (Liu, Yang et al. 2017). For municipal wastewater treatment, oxygen-limited condition created through intermittent aeration has been identified as the dominant and preferred mechanism of NOB suppression because DO within the system can be easily controlled via manipulating air supply. However, maintaining long-term partial nitrification process in biofilm-based reactors like the MABR can be challenging due to the long solids retention times (SRT) which can makes NOB vulnerable to acclimatization to low DO condition (Wett, Omari et al. 2013). Limited studies have been published on nitrogen removal from municipal wastewater via nitritation using MABR technology due to the low level of ammonium in municipal wastewater which suppresses AOB specific growth rate. However, studies on nitritation coupled with anaerobic ammonium oxidation (Anammox) is currently gaining much attention.

Summary of recent MABR-PND research papers is presented in Table A1.

2.9.4. Partial nitrification /anammox (PNA)

Of recent interest is the removal of nitrogen from wastewater via the anaerobic ammonium oxidation (anammox) pathway. In the anammox process, AOBs oxidizes 50% of the ammonia in wastewater to nitrite under aerobic condition (eqn. 5) (partial nitrification), while anaerobic ammonia oxidizing bacteria (AnAOB) oxidizes the remaining ammonia to nitrogen gas using nitrite as the electron acceptor (eqn. 6). Eqn. 7 shows the overall PNA stoichiometric relationship, partial nitrification, provides stable and efficient nitrite for the process (Liu, Kim et al. 2020).

NOBs, are undesirable in the anammox process as they compete with AnAOB for nitrite and with AOB for oxygen (Wang, Liang et al. 2021). Micro-aerobic conditions that result in NOB suppression or complete cessation are required for anammox. it is expected that the synergy between partial-nitrification and anammox will facilitate cost-efficient nitrogen removal from wastewaters, as this combination have the advantages of > 50% reduction in aeration and carbon demand and >20 % carbon dioxide (CO_2) and sludge production (Wang, Xu et al. 2019, Liu, Kim et al. 2020).

Nitrogen removal via PNA pathway has been intensively studied using different systems, but investigations using MABR have mostly been limited to high-strength wastewater until recently (Lackner, Terada et al. 2010, Bunse, Orschler et al. 2020, Mehrabi, Houweling and Dagnew 2020).

2.9.5. CANDO

CANDO is another emerging and attractive process for nitrogen removal and energy recovery from wastewater. It is a 3-step process comprising of; partial nitrification ($NH_4^+ \rightarrow NO_2^-$), partial anoxic reduction of nitrite to nitrous oxide ($NO_2^- \rightarrow N_2O$), and nitrous oxide conversion to nitrogen gas ($N_2O \rightarrow N_2$) with energy recovery. The initial and final steps of the CANDO process have successfully been demonstrated at full scale, but the intermediate step is yet to be established, however, it is expected that if efficiently developed and optimized, CANDO could theoretically

decrease deammonification process oxygen requirements by 20%, decrease biomass production by 40%, and increase energy production by 60% despite high organic carbon requirement for the intermediate step (Scherson, Wells et al. 2013). However, the collection method of nitrous oxide remains concerning as nitrous oxide is the third most important Greenhouse gas (GHG) in terms of current radiative forcing, and its harmful effect on the ozone layer. Nitrous oxide possesses 298 times greater global warming potential than that of carbon dioxide (Ming, De_richter et al. 2016).

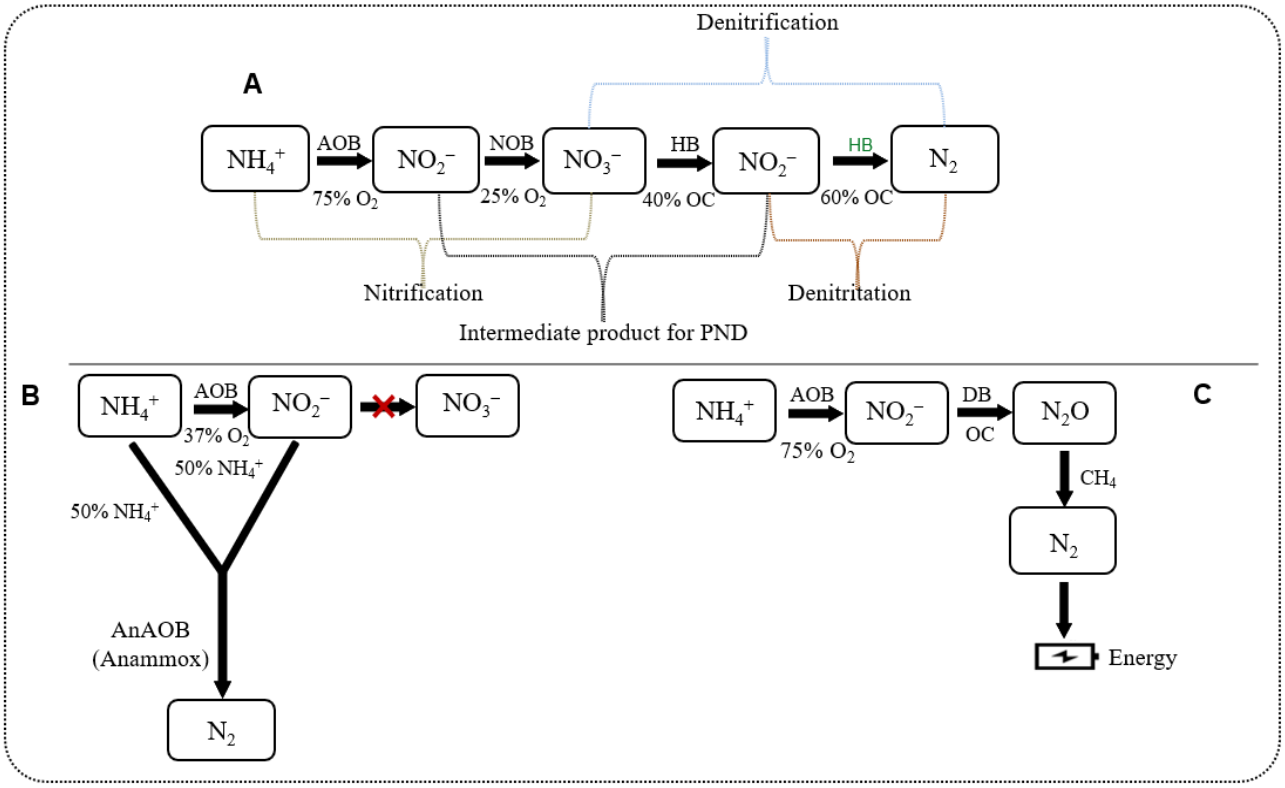


Figure 2.8. Nitrogen removal pathways in MABR. A) Complete nitrification and denitrification (ND), simultaneous nitrification-denitrification (SND), partial nitrification-denitrification (PND), B) Partial nitrification /anammox (PN/A), C) Coupled aerobic-anoxic nitrous decomposition operation (CANDO)

2.10. MABR low aeration energy potential

MABR remains a technology of interest, given its exceptionally low aeration energy potential (Heffernan et al., 2017), which is particularly significant amid the increasing focus on reducing wastewater treatment energy, a crucial aspect in the global pursuit of a decarbonized society. Many studies have successfully demonstrated this potential. Aybar et al., (2015) showed up to 85% electrical energy and 82 – 86% power savings when comparing MABR to CAS systems using fine-bubble diffusers. An improvement attributed to the superior oxygen transfer efficiency of the MABR membranes compared to fine-bubble diffusers. Fine-bubble diffusers are considered to have the highest energy efficiency in CAS systems (Heffernan et al., 2017). Similarly, in two case studies, Tirosh and Shechter (2020) reported a theoretical energy savings of 86.8% for MABR over CAS, for municipal wastewater and high-strength wastewater treatments respectively. Uri-Carreño et al. (2021) also reported high average aeration energy reduction of 74% in MABR compared to CAS using surface aerators. Moreover, the use of advanced aeration strategy, like intermittent aeration, has the potential to further enhance MABR energy saving (Uri-Carreño et al. 2021). Comparison of MABR technology energy consumption with similar technologies for municipal wastewater treatment is shown in Table 2.3.

Table 2.3: Aeration Energy Estimate for ***Municipal Wastewater Treatment Using Different Technologies***

Technology	Costs		Comment	Reference
	Electrical energy consumption per unit of treated wastewater (kWh/m3)	**Energy consumption per unit COD removed (kWh/kg COD)**		
MABR	0.05 – 0.1	0.15 – 0.4	4 times more efficient than CAS	Syron and Casey 2008 Syron and Heffernan, 2017 Tirosh and Shechter, 2020
CAS[a]	0.13 – 2.28	1.01–1.54	Standard for comparison	Liu et al., 2019, Gude, 2015 Siatou et al., 2020 Soares et al., 2017
MBR[b]	0.45 – 0.91	1.40 – 2.76	2 times more energy consumed than CAS but with potential for 3- 50 % reduction with newer technology	Krzeminski, et al., 2017
IFAS[c]	0.09 – 0.25	0.40 – 2.19	Energy use higher than CAS as coarse bubble aeration is used to keep media in suspension	Saghafi et al., 2016
AnMBR[d]	0.00186 – 5.68	~ 0.7	No aeration, but energy is required for gas sparging to reduce membrane fouling	Mahmood et al., 2022 McCarty, et al., 2011 Lee and Liao., 2021

a = Conventional activated sludge b = Membrane bioreactor c = Integrated fixed film activated sludge d = anaerobic membrane bioreactor

2.11. MABR microbial community

Studies have demonstrated that MABR bulk liquid and biofilm house multifunctional strata of microbial community containing diverse bacterial groups with distinct functions, including; nitrifying bacteria (AOBs (*Nitrosomonas* and *Nitrosospira*) and NOBs (*Nitrospira* and *Nitrobacte*r), denitrifying bacteria (DNB), anammox bacteria, polyphosphate accumulating organisms (PAOs) *etc.*, depending on operating parameters and conditions (Tian, Zhao et al. 2015, Li and Liu 2019, Mehrabi, Houweling and Dagnew 2020, Zhou, Li et al. 2020). Nitrifiers are preferentially located in the oxygen-rich region adjacent to the membrane-biofilm interface, whereas denitrifiers grow in the anoxic region at the biofilm-liquid interface where the COD concentration is typically at

maximum (Syron and Casey 2008, Nerenberg 2016). Several investigators have confirmed microbial community stratification in MABR biofilm by comparing the population of nitrifiers and denitrifies at the different parts of the biofilm (Yamagiwa, Ohkawa and Hirasa 1994, Downing and Nerenberg 2008, Ma, Domingo-Felez et al. 2017). MABR biofilm microbial community stratification has also been explored using model studies (Shanahan 2007, Downing and Nerenberg 2008).

However, the traditional two-step ammonia removal from wastewater by two distinct nitrifiers (AOB and NOB) has recently been challenged with the discovery of complete ammonia oxidation (comammox, CMX) *Nitrospira,* a single micro-organism, belonging to the genus *Nitrospira*. CMX *Nitrospira* contains ammonia oxidation and nitrite oxidation genes and consequently has the capacity to convert NH_4^+-N directly to NO_3^- (Lawson and Lücker 2018). CMX *Nitrospira* also have other advantages, including; high growth yield and high adaptive capacity to nutrient and growth-limiting conditions (Maddela, Gan et al. 2022). High relative abundance and dominance of CMX have been widely reported in wastewater treatment plants, and nitrifying activated sludge systems (Maddela, Gan et al. 2022). However, full-fledged WWTPs operations with CMX bacteria have not been reported, as several key aspects of the bacteria such as, interactions with other bacteria, reactor adaptability, stress response, and co-metabolic biotransformation are yet to be understood (Spasov, Tsuji et al. 2020, Maddela, Gan et al. 2022). Comammox and anammox metabolic pathways for nitrogen removal from wastewater were first observed in biofilm reactors (Boltz, Smets et al. 2017) however, limited MABR studies have reported nitrogen removal through comammox pathway.

Pollutants' removal efficiency of the MABR, depends on adequate balance between the activities of the different microbial groups within the biofilm. However, MABR biofilm microbial community structure still remains unclear, making it an area of high research interest (Schramm, De

Beer et al. 2000, Liu, Tan et al. 2014, Tian, Zhao et al. 2015). Considerable research efforts are currently being invested into studies geared towards the understanding of the overall biodiversity of MABR microbial community in terms of properties, functions and activities (Ontiveros-Valencia, Zhou et al. 2018).

2.12. References

Ahmed, T. and M. J. Semmens (1992). "Use of sealed end hollow fibers for bubbleless membrane aeration: experimental studies." Journal of Membrane Science **69**(1-2): 1-10.

Aybar, M., G. Pizarro, J. Boltz, L. Downing and R. Nerenberg (2014). "Energy-efficient wastewater treatment via the air-based, hybrid membrane biofilm reactor (hybrid MfBR)." Water science and technology **69**(8): 1735-1741.

Aybar, M., G. Pizarro, J. Boltz and R. Nerenberg (2015). Energy efficient wastewater treatment via the membrane-aerated biofilm reactor (MABR): Effect of membrane fluxes on performance. WEFTEC 2015, Water Environment Federation.

Blackburne, R., Z. G. Yuan and J. Keller (2008). "Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor." Biodegradation. **19**.

Boltz, J. P., B. F. Smets, B. E. Rittmann, M. C. Van Loosdrecht, E. Morgenroth and G. T. Daigger (2017). "From biofilm ecology to reactors: a focused review." Water Science and Technology **75**(8): 1753-1760.

Bunse, P., L. Orschler, S. Agrawal and S. Lackner (2020). "Membrane aerated biofilm reactors for mainstream partial nitritation/anammox: Experiences using real municipal wastewater." Water Research X **9**: 100066.

Cao, C., L. Zhao, D. Xu and Q. Geng (2009). "Membrane-aerated biofilm reactor behaviors for the treatment of high-strength ammonium industrial wastewater." Chemical Engineering & Technology: Industrial Chemistry-Plant Equipment-Process Engineering-Biotechnology **32**(4): 613-621.

Carlson, A. L., H. He, C. Yang and G. T. Daigger (2021). "Comparison of hybrid membrane aerated biofilm reactor (MABR)/suspended growth and conventional biological nutrient removal processes." Water Science and Technology **83**(6): 1418-1428.

Carniello, V., B. W. Peterson, H. C. van der Mei and H. J. Busscher (2018). "Physico-chemistry from initial bacterial adhesion to surface-programmed biofilm growth." Advances in colloid and interface science **261**: 1-14.

Casey, E., B. Glennon and G. Hamer (1999). "Oxygen mass transfer characteristics in a membrane-aerated biofilm reactor." Biotechnology and bioengineering **62**(2): 183-192.

Casey, E., B. Glennon and G. Hamer (2000). "Biofilm development in a membrane-aerated biofilm reactor: Effect of flow velocity on performance." Biotechnology and Bioengineering **67**(4): 476-486.

Castagna, L., A. Zanella, V. Scaravilli, F. Magni, S. A. E. A. E. S. Deab, M. Introna, F. Mojoli, G. Grasselli, A. Pesenti and N. Patroniti (2015). "Effects on membrane lung gas exchange of an intermittent high gas flow recruitment maneuver: preliminary data in veno-venous ECMO patients." Journal of Artificial Organs **18**: 213-219.

Castrillo, M., R. Díez-Montero, A. L. Esteban-García and I. Tejero (2019). "Mass transfer enhancement and improved nitrification in MABR through specific membrane configuration." Water research **152**: 1-11.

Chai, H., Y. Xiang, R. Chen, Z. Shao, L. Gu, L. Li and Q. He (2019). "Enhanced simultaneous nitrification and denitrification in treating low carbon-to-nitrogen ratio wastewater: Treatment performance and nitrogen removal pathway." Bioresource technology **280**: 51-58.

Chen, R., S. Cao, L. Zhang and Y. Zhou (2022). "NOB suppression strategies in a mainstream membrane aerated biofilm reactor under exceptionally low lumen pressure." Chemosphere **290**: 133386.

Cole, A. C., M. J. Semmens and T. M. LaPara (2004). "Stratification of activity and bacterial community structure in biofilms grown on membranes transferring oxygen." Applied and Environmental Microbiology **70**(4): 1982-1989.

Cole, A. C., J. W. Shanahan, M. J. Semmens and T. M. LaPara (2002). "Preliminary studies on the microbial community structure of membrane-aerated biofilms treating municipal wastewater." Desalination **146**(1-3): 421-426.

Corsino, S. F. and M. Torregrossa (2022). "Achieving complete nitrification below the washout SRT with hybrid membrane aerated biofilm reactor (MABR) treating municipal wastewater." Journal of Environmental Chemical Engineering **10**(1): 106983.

Cote, P., J.-L. Bersillon, A. Huyard and G. Faup (1988). "Bubble-free aeration using membranes: process analysis." Journal (Water Pollution Control Federation): 1986-1992.

Côté, P., J. Peeters, N. Adams, Y. Hong, Z. Long and J. Ireland (2015). A new membrane-aerated biofilm reactor for low energy wastewater treatment: pilot results. WEFTEC 2015, Water Environment Federation.

Côté, P., J. Peeters, N. Adams, Y. Hong, Z. Long and J. Ireland (2015). "A new membrane-aerated biofilm reactor for low energy wastewater treatment: Pilot results." Proceedings of the Water Environment Federation **13**: 4226-4239.

Da Silva, T. S., T. Matsumoto, M. L. Dos Anjos and L. L. Albertin (2018). "Organic matter removal in a membrane-aerated biofilm reactor." Journal of Environmental Engineering **144**(8): 04018057.

De Meis, D. (2017). Gas transport through porous membranes, ENEA.

Denkhaus, E., S. Meisen, U. Telgheder and J. Wingender (2007). "Chemical and physical methods for characterisation of biofilms." Microchimica Acta **158**: 1-27.

Dong, W.-y., H.-j. Wang, W.-g. Li, W.-c. Ying, G.-h. Gan and Y. Yang (2009). "Effect of DO on simultaneous removal of carbon and nitrogen by a membrane aeration/filtration combined bioreactor." Journal of Membrane Science **344**(1-2): 219-224.

Downing, L. S. and R. Nerenberg (2008). "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.

Elsayed, A., M. Hurdle and Y. Kim (2021). "Comprehensive model applications for better understanding of pilot-scale membrane-aerated biofilm reactor performance." Journal of Water Process Engineering **40**: 101894.

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

Grady Jr, C. L., G. T. Daigger, N. G. Love and C. D. Filipe (2011). Biological wastewater treatment, CRC press.

Gude, V. G. (2015). "Energy and water autarky of wastewater treatment and power generation systems." Renewable and sustainable energy reviews **45**: 52-68.

Guglielmi, G., D. Coutts, D. Houweling and J. Peeters (2020). "FULL-SCALE APPLICATION OF MABR TECHNOLOGY FOR UPGRADING AND RETROFITTING AN EXISTING WWTP: PERFORMANCES AND PROCESS MODELLING." Environmental Engineering & Management Journal (EEMJ) **19**(10).

He, H., B. M. Wagner, A. L. Carlson, C. Yang and G. T. Daigger (2021). "Recent progress using membrane aerated biofilm reactors for wastewater treatment." Water Science and Technology **84**(9): 2131-2157.

Heffernan, B., A. Shrivastava, D. Toniolo, M. Semmens and E. Syron (2017). Operation of a large scale membrane aerated biofilm reactor for the treatment of municipal wastewater. WEFTEC 2017, Water Environment Federation.

Heffernan, B., A. Shrivastava, D. Toniolo, M. Semmens and E. Syron (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.

Hibiya, K., A. Terada, S. Tsuneda and A. Hirata (2003). "Simultaneous nitrification and denitrification by controlling vertical and horizontal microenvironment in a membrane-aerated biofilm reactor." Journal of biotechnology **100**(1): 23-32.

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

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

Houweling, D. and G. T. Daigger (2019). Intensifying Activated Sludge Using Media-Supported Biofilms, CRC Press.

Houweling, D., J. Peeters, P. Cote, Z. Long and N. Adams (2017). "Proving membrane aerated biofilm reactor (MABR) performance and reliability: Results from four pilots and a full-scale plant." Proceedings of the Water Environment Federation **2017**(16): 272-284.

Houweling, D., J. Peeters, P. Cote, Z. Long and N. Adams (2017). Proving membrane aerated biofilm reactor (MABR) performance and reliability: Results from four pilots and a full-scale plant. WEFTEC 2017, Water Environment Federation.

Hu, S., F. Yang, S. Liu and L. Yu (2009). "The development of a novel hybrid aerating membrane-anaerobic baffled reactor for the simultaneous nitrogen and organic carbon removal from wastewater." Water Research **43**(2): 381-388.

Hu, X. b., K. Xu, Z. Wang, L. l. Ding and H. q. Ren (2013). "Characteristics of biofilm attaching to carriers in moving bed biofilm reactor used to treat vitamin C wastewater." Scanning **35**(5): 283-291.

Hu, Z., D. Houweling and P. Dold (2012). "Biological nutrient removal in municipal wastewater treatment: new directions in sustainability." Journal of Environmental Engineering **138**(3): 307-317.

Janczewski, L. and A. Trusek-Holownia (2016). "Biofilm-based membrane reactors–selected aspects of the application and microbial layer control." Desalination and Water Treatment **57**(48-49): 22909-22916.

Kinh, C. T., S. Riya, M. Hosomi and A. Terada (2017). "Identification of hotspots for NO and N2O production and consumption in counter-and co-diffusion biofilms for simultaneous nitrification and denitrification." Bioresource technology **245**: 318-324.

Krzeminski, P., L. Leverette, S. Malamis and E. Katsou (2017). "Membrane bioreactors–a review on recent developments in energy reduction, fouling control, novel configurations, LCA and market prospects." Journal of Membrane Science **527**: 207-227.

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

Lackner, S., A. Terada, H. Horn, M. Henze and B. F. Smets (2010). "Nitritation performance in membrane-aerated biofilm reactors differs from conventional biofilm systems." Water Research **44**(20): 6073-6084.

Lai, C.-Y., L. Zhong, Y. Zhang, J.-X. Chen, L.-L. Wen, L.-D. Shi, Y.-P. Sun, F. Ma, B. E. Rittmann and C. Zhou (2016). "Bioreduction of chromate in a methane-based membrane biofilm reactor." Environmental science & technology **50**(11): 5832-5839.

Lawson, C. E. and S. Lücker (2018). "Complete ammonia oxidation: an important control on nitrification in engineered ecosystems?" Current opinion in biotechnology **50**: 158-165.

Lee, H. S. and B. Liao (2021). "Anaerobic membrane bioreactors for wastewater treatment: Challenges and opportunities." Water Environment Research **93**(7): 993-1004.

Lema, J. M. and S. S. Martinez (2017). Innovative wastewater treatment & resource recovery technologies: impacts on energy, economy and environment, IWA publishing.

Li, J., L.-P. Zhu, Y.-Y. Xu and B.-K. Zhu (2010). "Oxygen transfer characteristics of hydrophilic treated polypropylene hollow fiber membranes for bubbleless aeration." Journal of Membrane Science **362**(1-2): 47-57.

Li, M., C. Du, J. Liu, X. Quan, M. Lan and B. Li (2018). "Mathematical modeling on the nitrogen removal inside the membrane-aerated biofilm dominated by ammonia-oxidizing archaea (AOA): effects of temperature, aeration pressure and COD/N ratio." Chemical Engineering Journal **338**: 680-687.

Li, M., Y. Li, N. Wang, T. Li, H. Guo, Z. Wu, P. Zhang, B. Wang and B. Li (2022). "Achieving efficient nitrogen removal in a single-stage partial nitrification-anammox-partial denitrification (PN/A/PD) membrane aerated biofilm reactor (MABR)." Journal of Water Process Engineering **49**: 103100.

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

Li, X., D. Bao, Y. Zhang, W. Xu, C. Zhang, H. Yang, Q. Ru, Y.-f. Wang, H. Ma and E. Zhu (2023). "Development and application of membrane aerated biofilm reactor (MABR)—A review." Water **15**(3): 436.

Li, Y. and K. Zhang (2018). "Pilot scale treatment of polluted surface waters using membrane-aerated biofilm reactor (MABR)." Biotechnology & Biotechnological Equipment **32**(2): 376-386.

Liao, B. and S. Liss (2007). "A comparative study between thermophilic and mesophilic membrane aerated biofilm reactors." Journal of Environmental Engineering and Science **6**(2): 247-252.

Lin, J., P. Zhang, J. Yin, X. Zhao and J. Li (2015). "Nitrogen removal performances of a polyvinylidene fluoride membrane-aerated biofilm reactor." International Biodeterioration & Biodegradation **102**: 49-55.

Liu, Y., J. Gu and M. Zhang (2019). AB processes: Towards energy self-sufficient municipal wastewater treatment, IWA publishing.

Liu, H., S. Tan, Z. Sheng, Y. Liu and T. Yu (2014). "Bacterial community structure and activity of sulfate reducing bacteria in a membrane aerated biofilm analyzed by microsensor and molecular techniques." Biotechnology and Bioengineering **111**(11): 2155-2162.

Liu, H., F. Yang, T. Wang, Q. Liu and S. Hu (2007). "Carbon membrane-aerated biofilm reactor for synthetic wastewater treatment." Bioprocess and biosystems engineering **30**(4): 217-224.

Liu, W., Q. Yang, B. Ma, J. Li, L. Ma, S. Wang and Y. Peng (2017). "Rapid achievement of nitritation using aerobic starvation." Environmental Science & Technology **51**(7): 4001-4008.

Liu, X., M. Kim, G. Nakhla, M. Andalib and Y. Fang (2020). "Partial nitrification-reactor configurations, and operational conditions: Performance analysis." Journal of Environmental Chemical Engineering **8**(4): 103984.

Lu, D., H. Bai, F. Kong, S. N. Liss and B. Liao (2021). "Recent advances in membrane aerated biofilm reactors." Critical Reviews in Environmental Science and Technology **51**(7): 649-703.

Lu, J.-J., Q. Shen, X.-Y. Li, F.-Y. Sun, J.-B. Yi, W.-Y. Dong, W.-J. Yan, H. Du and J.-L. Mu (2022). "Surface-manipulated membranes to accelerate biofilm formation and to resist bacterial detachment in MBfR for aerobic methane oxidation coupled to denitrification." Chemical Engineering Journal **430**: 132629.

Ma, Y., C. Domingo-Felez, B. G. Plósz and B. F. Smets (2017). "Intermittent aeration suppresses nitrite-oxidizing bacteria in membrane-aerated biofilms: a model-based explanation." Environmental science & technology **51**(11): 6146-6155.

Ma, Y., A. Piscedda, A. D. L. C. Veras, C. Domingo-Félez and B. F. Smets (2022). "Intermittent aeration to regulate microbial activities in membrane-aerated biofilm reactors: Energy-efficient nitrogen removal and low nitrous oxide emission." Chemical Engineering Journal **433**: 133630.

Maddela, N. R., Z. Gan, Y. Meng, F. Fan and F. Meng (2022). "Occurrence and roles of comammox bacteria in water and wastewater treatment systems: a critical review." Engineering **17**: 196-206.

Mahmood, Z., H. Cheng and M. Tian (2022). "A critical review on advanced anaerobic membrane bioreactors (AnMBR) for wastewater treatment: advanced membrane materials and energy demand." Environmental Science: Water Research & Technology.

Martin, K. J., J. P. Boltz and R. Nerenberg (2012). The Membrane Biofilm Reactor (MBfR) for Wastewater Treatment: Applications, Design Considerations, and Technology Outlook. Proceedings of 85th Annual Water Environment Federation Technical Exposition & Conference.

Martin, K. J. and R. Nerenberg (2012). "The membrane biofilm reactor (MBfR) for water and wastewater treatment: principles, applications, and recent developments." Bioresource technology **122**: 83-94.

McCarty, P. L., J. Bae and J. Kim (2011). Domestic wastewater treatment as a net energy producer–can this be achieved?, ACS Publications.

Mehrabi, S., D. Houweling and M. Dagnew (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.

Melo, L. F. (2003). "Biofilm formation and its role in fixed film processes." book: Water Wastewater Microbiology: 337-349.

Ming, T., R. De_richter, S. Shen and S. Caillol (2016). "Fighting global warming by greenhouse gas removal: destroying atmospheric nitrous oxide thanks to synergies between two breakthrough technologies." Environmental Science and Pollution Research **23**: 6119-6138.

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

Nerenberg, R. (2005). Membrane biofilm reactors for water and wastewater treatment. Borchardt Conference: A Seminar on Advances in Water and Wastewater Treatment, Ann Arbor. MI.

Nerenberg, R. (2016). "The membrane-biofilm reactor (MBfR) as a counter-diffusional biofilm process." Current opinion in biotechnology **38**: 131-136.

Nisola, G. M., J. Orata-Flor, S. Oh, N. Yoo and W.-J. Chung (2013). "Partial nitrification in a membrane-aerated biofilm reactor with composite PEBA/PVDF hollow fibers." Desalination and Water Treatment **51**(25-27): 5275-5282.

Ohandja, D.-G. and D. C. Stuckey (2007). "Biodegradation of PCE in a hybrid membrane aerated biofilm reactor." Journal of Environmental Engineering **133**(1): 20-27.

Ontiveros-Valencia, A., C. Zhou, H.-P. Zhao, R. Krajmalnik-Brown, Y. Tang and B. Rittmann (2018). "Managing microbial communities in membrane biofilm reactors." Applied microbiology and biotechnology **102**: 9003-9014.

Pankhania, M., T. Stephenson and M. J. Semmens (1994). "Hollow fibre bioreactor for wastewater treatment using bubbleless membrane aeration." Water research **28**(10): 2233-2236.

Park, S., W. Bae and B. E. Rittmann (2010). "Operational boundaries for nitrite accumulation in nitrification based on minimum/maximum substrate concentrations that include effects of oxygen limitation, pH, and free ammonia and free nitrous acid inhibition." Environmental science & technology **44**(1): 335-342.

Paul, E., J. C. Ochoa, Y. Pechaud, Y. Liu and A. Liné (2012). "Effect of shear stress and growth conditions on detachment and physical properties of biofilms." Water research **46**(17): 5499-5508.

Peeters, J., Z. Long, D. Houweling, P. Côté, G. T. Daigger and S. Snowling (2017). Nutrient removal intensification with MABR–developing a process model supported by piloting. Nutrient Removal and Recovery Symposium 2017, Water Environment Federation.

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

Perez-Calleja, P., M. Aybar, C. Picioreanu, A. L. Esteban-Garcia, K. J. Martin and R. Nerenberg (2017). "Periodic venting of MABR lumen allows high removal rates and high gas-transfer efficiencies." Water research **121**: 349-360.

Pérez-Calleja, P., E. Clements and R. Nerenberg (2022). "Enhancing ammonium oxidation fluxes and nitritation efficiencies in MABRs: a modeling study." Environmental Science: Water Research & Technology **8**(2): 358-374.

Rittmann, B. E. and P. L. McCarty (1980). "Model of steady-state-biofilm kinetics." Biotechnology and bioengineering **22**(11): 2343-2357.

Rothemund, C., A. K. Camper and P. Wilderer (1994). "Biofilms growing on gas permeable membranes."

Saghafi, S., G. R. Nabi Bid Hendy and H. Aminirad (2016). "Estimating the electrical energy in different processes for Nasir Abad industrial wastewater treatment plant with emphasis on COD removal." Journal of Environmental Studies **42**(1): 19-31.

Salbitani, G. and S. Carfagna (2021). "Ammonium utilization in microalgae: A sustainable method for wastewater treatment." Sustainability **13**(2): 956.

Sanchez-Huerta, C., L. Fortunato, T. Leiknes and P.-Y. Hong (2022). "Influence of biofilm thickness on the removal of thirteen different organic micropollutants via a Membrane Aerated Biofilm Reactor (MABR)." Journal of Hazardous Materials **432**: 128698.

Scherson, Y. D., G. F. Wells, S.-G. Woo, J. Lee, J. Park, B. J. Cantwell and C. S. Criddle (2013). "Nitrogen removal with energy recovery through N 2 O decomposition." Energy & Environmental Science **6**(1): 241-248.

Schramm, A., D. De Beer, A. Gieseke and R. Amann (2000). "Microenvironments and distribution of nitrifying bacteria in a membrane-bound biofilm." Environmental microbiology **2**(6): 680-686.

Sehar, S. and I. Naz (2016). "Role of the biofilms in wastewater treatment." Microbial biofilms-importance and applications: 121-144.

Semmens, M. (2005). "Membrane technology: pilot studies of membrane-aerated bioreactors."

Semmens, M. and D. Hanus (1999). "Studies of a membrane aerated bioreactor for wastewater treatment." Membrane Technology **1999**(111): 9-13.

Semmens, M. and J. Shanahan (2005). "Membrane Technology: Pilot Studies of Membrane Aerated Bioreactors, Final Report." Water Environment Research Foundation, Virginia, USA.

Semmens, M. J., K. Dahm, J. Shanahan and A. Christianson (2003). "COD and nitrogen removal by biofilms growing on gas permeable membranes." Water Research **37**(18): 4343-4350.

Shanahan, J. and M. Semmens (2006). "Influence of a nitrifying biofilm on local oxygen fluxes across a micro-porous flat sheet membrane." Journal of Membrane Science **277**(1-2): 65-74.

Shanahan, J. W. (2007). Modeling and Experimental Analyses of Membrane-aerated Biofilms, University of Minnesota.

Shanahan, J. W. and M. J. Semmens (2015). "Alkalinity and pH effects on nitrification in a membrane aerated bioreactor: an experimental and model analysis." Water Research **74**: 10-22.

Siatou, A., A. Manali and P. Gikas (2020). "Energy consumption and internal distribution in activated sludge wastewater treatment plants of Greece." Water **12**(4): 1204.

Silveira, I. T., K. Cadee and W. Bagg (2022). "Startup and initial operation of an MLE-MABR treating municipal wastewater." Water Science and Technology **85**(4): 1155-1166.

Soares, R. B., M. S. Memelli, R. P. Roque and R. F. Gonçalves (2017). "Comparative analysis of the energy consumption of different wastewater treatment plants." International Journal of Architecture, Arts and Applications **3**(6): 79-86.

Spasov, E., J. M. Tsuji, L. A. Hug, A. C. Doxey, L. A. Sauder, W. J. Parker and J. D. Neufeld (2020). "High functional diversity among Nitrospira populations that dominate rotating biological contactor microbial communities in a municipal wastewater treatment plant." The ISME Journal **14**(7): 1857-1872.

Stricker, A. E., H. Lossing, J. H. Gibson, Y. Hong and J. C. Urbanic (2011). "Pilot Scale Testing of A New Configuration of The Membrane Aerated Biofilm Reactor (MABR) to Treat High-Strength Industrial Sewage." Water Environment Research **83**(1): 3-14.

Suarez, C., M. Piculell, O. Modin, S. Langenheder, F. Persson and M. Hermansson (2019). "Thickness determines microbial community structure and function in nitrifying biofilms via deterministic assembly." Scientific reports **9**(1): 1-10.

Sun, L., Z. Wang, X. Wei, P. Li, H. Zhang, M. Li, B. Li and S. Wang (2015). "Enhanced biological nitrogen and phosphorus removal using sequencing batch membrane-aerated biofilm reactor." Chemical Engineering Science **135**: 559-565.

Sun, Z., M. Li, G. Wang, X. Yan, Y. Li, M. Lan, R. Liu and B. Li (2020). "Enhanced carbon and nitrogen removal in an integrated anaerobic/anoxic/aerobic-membrane aerated biofilm reactor system." RSC advances **10**(48): 28838-28847.

Sunner, N., Z. Long, D. Houweling, A. Monti and J. Peeters (2018). MABR as a low-energy compact solution for nutrient removal upgrades—results from a demonstration in the UK. WEFTEC 2018, Water Environment Federation.

Suzuki, Y., N. Hatano, S. Ito and H. Ikeda (2000). "Performance of nitrogen removal and biofilm structure of porous gas permeable membrane reactor." Water science and technology **41**(4-5): 211-217.

Syron, E. and E. Casey (2008). "Membrane-aerated biofilms for high rate biotreatment: performance appraisal, engineering principles, scale-up, and development requirements." Environmental science & technology **42**(6): 1833-1844.

Syron, E. and B. Heffernan (2017). OxyMem, The Flexiable MABR. Nutrient Removal and Recovery Symposium 2017, Water Environment Federation.

Syron, E., M. J. Semmens and E. Casey (2015). "Performance analysis of a pilot-scale membrane aerated biofilm reactor for the treatment of landfill leachate." Chemical Engineering Journal **273**: 120-129.

Taşkan, B., H. Hasar and C. H. Lee (2020). "Effective biofilm control in a membrane biofilm reactor using a quenching bacterium (Rhodococcus sp. BH4)." Biotechnology and Bioengineering **117**(4): 1012-1023.

Terada, A., K. Hibiya, J. Nagai, S. Tsuneda and A. Hirata (2003). "Nitrogen removal characteristics and biofilm analysis of a membrane-aerated biofilm reactor applicable to high-strength nitrogenous wastewater treatment." Journal of bioscience and bioengineering **95**(2): 170-178.

Terada, A., T. Yamamoto, R. Igarashi, S. Tsuneda and A. Hirata (2006). "Feasibility of a membrane-aerated biofilm reactor to achieve controllable nitrification." Biochemical engineering journal **28**(2): 123-130.

Tian, H.-L., J.-Y. Zhao, H.-Y. Zhang, C.-Q. Chi, B.-A. Li and X.-L. Wu (2015). "Bacterial community shift along with the changes in operational conditions in a membrane-aerated biofilm reactor." Applied microbiology and biotechnology **99**: 3279-3290.

Tian, H., H. Zhang, P. Li, L. Sun, F. Hou and B. Li (2015). "Treatment of pharmaceutical wastewater for reuse by coupled membrane-aerated biofilm reactor (MABR) system." RSC advances **5**(85): 69829-69838.

Timberlake, D. L., S. E. Strand and K. J. Williamson (1988). "Combined aerobic heterotrophic oxidation, nitrification and denitrification in a permeable-support biofilm." Water research **22**(12): 1513-1517.

Tirosh, U. and R. Shechter (2020). Membrane aerated biofilm reactor (MABR)—Distributed treatment of wastewater at low energy consumption. Frontiers in Water-Energy-Nexus—Nature-Based Solutions, Advanced Technologies and Best Practices for Environmental Sustainability: Proceedings of the 2nd WaterEnergyNEXUS Conference, November 2018, Salerno, Italy, Springer.

Ukaigwe, S., Y. Zhou, M. Shaheen and Y. Liu (2021). "Municipal wastewater treatment using a membrane aerated biofilm reactor." Journal of Environmental Engineering and Science **17**(2): 99-107.

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

Uri-Carreño, N., P. H. Nielsen, K. V. Gernaey and X. Flores-Alsina (2021). "Long-term operation assessment of a full-scale membrane-aerated biofilm reactor under Nordic conditions." Science of the Total Environment **779**: 146366.

Verstraete, W. and S. E. Vlaeminck (2011). "ZeroWasteWater: short-cycling of wastewater resources for sustainable cities of the future." International Journal of Sustainable Development & World Ecology **18**(3): 253-264.

Wagner, B., G. Daigger and N. Love (2022). "Assessing membrane aerated biofilm reactor configurations in mainstream anammox applications." Water Science and Technology **85**(3): 943-960.

Wang, H., G. Xu, Z. Qiu, Y. Zhou and Y. Liu (2019). "NOB suppression in pilot-scale mainstream nitritation-denitritation system coupled with MBR for municipal wastewater treatment." Chemosphere **216**: 633-639.

Wang, J., J. Liang, L. Sun, J. Shen and M. Wang (2021). "Achieving reliable partial nitrification and anammox process using polyvinyl alcohol gel beads to treat low-strength ammonia wastewater." Bioresource technology **324**: 124669.

Wett, B., A. Omari, S. Podmirseg, M. Han, O. Akintayo, M. Gómez Brandón, S. Murthy, C. Bott, M. Hell and I. Takács (2013). "Going for mainstream deammonification from bench to full scale for maximized resource efficiency." Water science and technology **68**(2): 283-289.

Wu, J. and V. Chen (2000). "Shell-side mass transfer performance of randomly packed hollow fiber modules." Journal of Membrane Science **172**(1-2): 59-74.

Wu, Y., Z. Wu, H. Chu, J. Li, H. H. Ngo, W. Guo, N. Zhang and H. Zhang (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.

Xia, S., F. Zhong, Y. Zhang, H. Li and X. Yang (2010). "Bio-reduction of nitrate from groundwater using a hydrogen-based membrane biofilm reactor." Journal of Environmental Sciences **22**(2): 257-262.

Xiao, P., J. Zhou, X. Luo, B. Kang, L. Guo, G. Yuan, L. Zhang and T. Zhao (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.

Yamagiwa, K., A. Ohkawa and O. Hirasa (1994). "Simultaneous organic carbon removal and nitrification by biofilm formed on oxygen enrichment membrane." Journal of chemical engineering of Japan **27**(5): 638-643.

Yang, Q., Y. Peng, X. Liu, W. Zeng, T. Mino and H. Satoh (2007). "Nitrogen removal via nitrite from municipal wastewater at low temperatures using real-time control to optimize nitrifying communities." Environmental science & technology **41**(23): 8159-8164.

Yang, S., S. Xu, A. Mohammed, B. Guo, S. Vincent, N. J. Ashbolt and Y. Liu (2019). "Anammox reactor optimization for the treatment of ammonium rich digestate lagoon supernatant-Step feeding mitigates nitrite inhibition." International biodeterioration & biodegradation **143**: 104733.

Yeh, S.-J. and C. R. Jenkins (1978). "Pure oxygen fixed film reactor." Journal of the Environmental Engineering Division **104**(4): 611-623.

Zhang, X. and Y. Liu (2022). "Circular economy is game-changing municipal wastewater treatment technology towards energy and carbon neutrality." Chemical Engineering Journal **429**: 132114.

Zheng, X., S. Zhang, J. Zhang, D. Huang and Z. Zheng (2018). "Advanced nitrogen removal from municipal wastewater treatment plant secondary effluent using a deep bed denitrification filter." Water Science and Technology **77**(11): 2723-2732.

Zhou, C., A. Ontiveros-Valencia, R. Nerenberg, Y. Tang, D. Friese, R. Krajmalnik-Brown and B. E. Rittmann (2019). "Hydrogenotrophic microbial reduction of oxyanions with the membrane biofilm reactor." Frontiers in microbiology **9**: 3268.

Zhou, Y., R. Li, B. Guo, L. Zhang, X. Zou, S. Xia and Y. Liu (2020). "Greywater treatment using an oxygen-based membrane biofilm reactor: Formation of dynamic multifunctional biofilm for organics and nitrogen removal." Chemical Engineering Journal **386**: 123989.

Zou, X., Y. Zhou, M. Gao, S. Yang, A. Mohammed and Y. Liu (2022). "Effective N2O emission control during the nitritation/denitritation treatment of ammonia rich wastewater." Journal of Environmental Chemical Engineering **10**(2): 107234.

Chapter 3 – Impact of Hydraulic Retention Time (HRT) on MABR Performance, Stability and Microbial Community Structure – Investigation Using Real Municipal Wastewater Primary Effluent

3.1. Introduction

Biological wastewater treatment using MABR employs a gas permeable membrane to deliver efficient microbe-assisted pollutant degradation. In the MABR, pressurized air (~21% O_2) is delivered through the membrane lumen to the biofilm that grows on the exterior of the membrane, domiciled in the wastewater. Pollutants oxidation occur inside the biofilm. Mass transfer of gas molecules and pollutants inside the biofilm move in reverse directions, thus, electron donors and electron acceptors enter the biofilm from opposing sides. Therefore, simultaneous nitrification, denitrification and the removal of organics are achievable in a single-stage MABR (Semmens and Hanus, 1999; Hibiya et al., 2003; Martins et al., 2012).

Using significantly lower energy and lower carbon footprint, an MABR can achieve the same effluent quality as conventional activated sludge process (Lema and Martinez, 2017), due to reductions in reactor area and volume requirements when treating large quantities of wastewater as a result of low HRT application. Although a minimum HRT is still required to accomplish the complete removal of specific pollutants.

[1]This minimum HRT is a function of both the pollutants' biodegradability index and process operating conditions like temperature, which influences the reaction kinetics. HRT affects both performance and microbial community dynamics within a biofilm. For instance, shorter HRT may

[1] A version of this chapter has been published in Journal of Environmental Engineering and Science as: Ukaigwe, Sandra Zhou, Yun Shaheen, Mohamed Liu, Yang "Municipal wastewater treatment using a membrane aerated biofilm reactor." *Journal of Environmental Engineering and Science,* 2021

eliminate active microbial populations thereby reducing performance (Grosser, 2017; Aybar et al., 2014b; Bui et al., 2019; Hu et al., 2008), while longer HRT increases operational costs.

This study was therefore focused on evaluating the impact of HRT on MABR performance, stability, microbial community structure and optimizing the HRT under mainstream conditions. Using graduated HRT changes, the impact of high and variable hydraulic loads on an MABR treating real municipal wastewater primary effluent (PE) was explored.

3.2. Materials and methods

3.2.1. Wastewater characteristics

Real municipal wastewater PE from a full-scale municipal wastewater treatment plant in Alberta, Canada was used for the study. The characteristics of the PE used in the five stages (200 days) of the study are shown in Table 3.1.

Table 3.1. Experimental Conditions and PE Characteristics

Stage	Time (d)	HRT [a] (h)	TCOD[b] (mg COD L^{-1})	TCOD Loading (g COD m^{-2} d^{-1})	sCOD[c] (mg L^{-1})	NH_4^+-N[d] (mg N L^{-1})	NH_4^+-N Loading (gN m^{-2} d^{-1})
Start up	1 -13	10.7	150 (58)	1.44 (0.56)	53.33 (9.95)	32.41 (2.01)	0.31 (0.03)
1	16 – 46	10.7	223 (37)	2.15 (0.36)	68.62 (16.66)	32.41 (2.01)	0.31 (0.03)
2	49 – 80	6.24	195 (80)	3.09 (1.26)	100 (30.28)	33.69 (3.71)	0.44 (0.14)
3	84 – 126	4	297 (41)	7.73 (1.06)	66.38 (24.45)	57.64 (3.02)	1.50 (0.07)
4	129 – 159	2	328 (41)	14.73 (3.93)	78.38 (29.64)	55.25 (7.18)	2.87 (0.42)
5	162 – 198	3	276 (29)	9.39 (0.97)	84.25 (20.92)	43.03 (6.30)	1.46 (0.21)

[a] HRT – hydraulic retention time, [b] TCOD – total chemical oxygen demand, [c] sCOD – soluble chemical oxygen demand.
[d] NH_4^+-N – ammonium-nitrogen. (Numbers in parentheses are the standard deviations of the average parameter values).

Table 3.2. Membrane Module and Reactor Parameters

Component	Unit	Value
Hollow fiber membrane length	m	0.23
Membrane outer diameter	mm	2.2
Membrane surface area	m^2	0.0156
Specific surface area	m^2m^{-3}	183.5
Membrane count	number	17
Reactor volume	ml	100
Working volume	ml	85
Reactor packing density	%	14.6

3.2.2. Experimental design

The MABR system was made up of two polystyrene plastic tubes – the larger tube was the main reactor and the smaller tube was the sampling tube. The tubes were connected with plastic fittings. The main reactor was connected with a module containing 20 membranes; each membrane was sealed at one end to produce a dead-end configuration. Both ends of the reactor were glued into an air-supply manifold, with a mechanism that allowed for periodic gas venting to prevent condensation of water vapour and other gases within the reactor. The sampling tube consisted of 10 membranes; each membrane was connected to the air-supply manifold at the top end and unconstrained at the other end. A hydrophobic microporous high gas-permeable hollow-fibre polyvinylidene difluoride (PVDF) membrane with a surface area of 0.042 m^2, a length of 230 mm, an outer diameter of 2.2 mm, and an average pore size of 0.1 μm was used in the main reactor and in the sampling tube. The reactor working volume was 200 mL; the net pool was 171.2 mL giving a packing density of 28.8%. Careful monitoring of the process was done to lower the risk associated with small reactor including sampling from a sample tube instead of the main reactor. Content uniformity for the reactors and detached biofilm reattachment along the membrane length were

achieved by recirculating the bulk liquid using a peristaltic pump (Longer Pump, BT100-2, Longer Precision Pump Co, Ltd., China) at a rate of 176 mL/min.

3.2.3. Reactor operation

The MABR was operated on a continuous basis for 200 days at ambient conditions with periodic venting. The reactor was inoculated with 5 mL aerobic activated sludge from the aeration tank of a municipal wastewater treatment plant. The values of the inoculum TSS and VSS were: 2.84 ± 0.12 and 2.07 ± 0.15 g/L, respectively. The influent pH ranged from 7.04 – 7.8. Influent feed was continuously pumped upward from the bottom of the reactor using a peristaltic pump. Air was supplied to the membrane lumen by an air pump and the air flow rate manually controlled with a valve and adjusted with a gas flow gauge. The intramembrane pressure was maintained at 2.0 psi (0.14 atm) throughout the experiments. A peristaltic pump (BT100-2 J, LongerPump®) with a recirculation rate of 12000 mL/h was used to recirculate the liquid between the two reactors to achieve complete mixing inside each reactor. Effluent was discharged by overflow from a tee fitting near the top of the sampling tube into a top-sealed 1 L glass bottle, and collected for parameter determination. Average temperature recorded during reactor operation was 20 ± 2 °C. The MABR was operated at flow rates of 7.96 – 42.5 mL/h, corresponding to HRTs of 2.0 h -10.7 h.

3.2.4. Parameter determination

The MABR performance was evaluated based on the types of pollutant removed, the pollutant removal efficiencies, and the rates of specific pollutant removal. These parameters were dependent on the operating conditions within the system; pH, aeration pressure and DO. The parameters measured in this study included influent and effluent concentrations of: TCOD, sCOD, NH_4^+-N, NO_3^-, NO_2^-, TN, pH and DO concentrations.

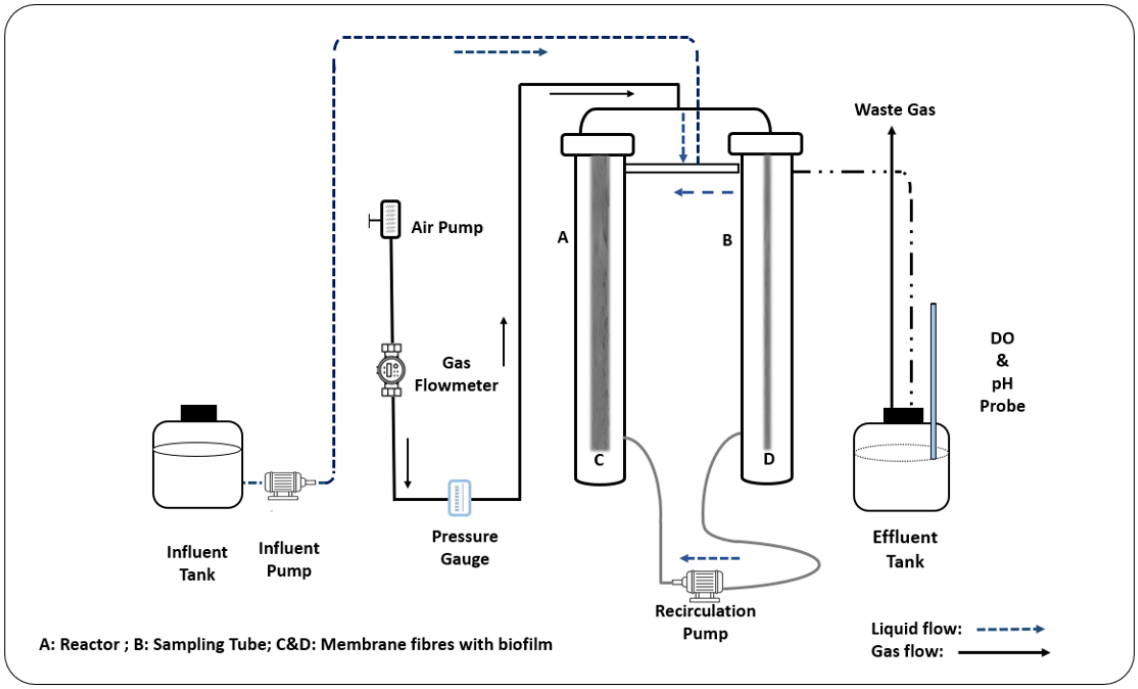


Figure 3.1. Process flow diagram of the lab-scale Membrane aerated biofilm reactor used in the study

3.2.5. Calculations

Pollutant removal efficiencies were estimated using equation (1); TCOD and NH_4^+-N surface loading rates were estimated using equation (2):

$$Rem.\ Eff. = \frac{Co\text{-}Ci}{Co} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$SLR = \frac{(Co\text{-}Ci)Q}{MSA} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where:

Co and *Ci* are influent and effluent pollutant concentrations (mg/L), respectively;

SLR is the surface loading rate (gTCOD m^{-2} d^{-1}; gNH_4^+-N m^{-2} d^{-1});

Q is the reactor influent flow rate (L d^{-1});

MSA is the membrane module surface area (m^2).

3.2.6. DNA extraction

Eight samples were collected: six biomass samples were collected from the membrane; one at the end of the start up stage and one after each of the five reaction stages—one sample was collected from the wastewater sludge, and one sample was collected from the tubing. Genomic DNA was extracted from approximately 0.5 g of each of the biofilm/sludge samples using Dneasy PowerSoil Kit (Qiagen Inc., Toronto, Canada) according to the manufacture's protocol. Polymerase chain reaction (PCR) was used to amplify the 16S rRNA genes using the universal primer pair 515F (GTGCCAGCMGCCGCGG) and the 806R (GGACTACHVGGGTWTCTAAT). DNA sample sequencing was performed on the Illumina Miseq PE250 platform at McGill University and the Génome Québec Innovation Centre (Montréal, Québec, Canada). The figure and statistical results were generated and analyzed using the microbiome analysis package QIIME 2 DADA2 pipeline with 99% similarity reference to the Greengenes database, version 13-8.

3.3. Results and Discussion

3.3.1. Removal of organics

Over the 200 days of operation, the process HRT was gradually reduced from 10.7 h – 2 h, corresponding to an increase in the surface loading rate from 0.81- 20.80 gTCOD m^{-2} d^{-1}. The HRT was increased to 3 h when the system became unstable at an HRT of 2 h. The effects on reactor performance of the graduated HRT reduction were evaluated. The influent TCOD concentration of the raw municipal wastewater PE varied throughout the study period, ranging from 103 to 400 mg/L.

However, consistently low average TCOD in the reactor effluent (< 50 mg/L) was obtained corresponding to average TCOD removal efficiency of 72% - 99% (Figure 3.2a). This is consistent with high COD removal that has often been reported for MABR (He et al, 2021). Reactor performance was consistent from HRT of 10.67 – 4 h. However, upon reduction in HRT from 4 – 2h, (increased surface load), TCOD removal efficiency decreased from 99 to 90%. But, the

performance recovered, and the removal efficiency increased to 98% when the HRT was increased to 3h (Figure 3.2b). The high performance obtained in this study can be attributed to high abundance of heterotrophs present in the matured and stable MABR biofilm, having been in operation for >200 days.

Considering, the continual variable hydraulic shocks to the MABR from raw municipal wastewater, the high organic surface loading rate >20 gTCODm-^{2}d-^{1}, and the low operating HRTs, this MABR performed better than MABRs reported in other studies (Table 3.3) that were operated under similar conditions. The high performance may be associated high biomass concentration in the MABR biofilm, dead-end membrane configuration and the periodic venting of the reactor both of which enhanced oxygen transfer rate (OTR) and the oxygen flux in the MABR (Perez-Calleja et al., 2017).

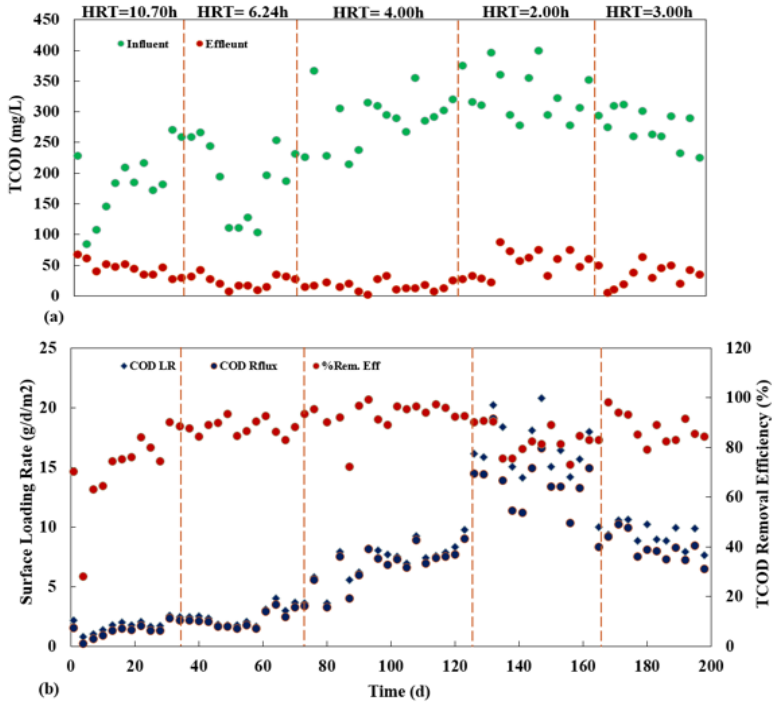


Figure 3.2. Reactor performance dynamics at each stage of the MABR operation. a) TCOD concentrations in influent and effluent. b) TCOD surface loading rates, removal flux, and removal efficiency.

3.3.2. Ammonia reduction

Influent NH_4^+-N concentrations fluctuated between 26 and 65 mg/L over the study period due to the variance in influent wastewater ammonia concentration; effluent NH_4^+-N concentrations ranged from 0.05 – 22.25 mg/L, corresponding to NH_4^+-N removal efficiencies of 64 – 99.9%. (Figure 3.3). The nitrification rate increased from 0.24 gN m^{-2} d^{-1} to a maximum of 3.12gN m^{-2} d^{-1} with the increase in influent ammonia concentration. In contrast to the TCOD removal efficiency, the NH_4^+-N removal efficiency increased with reduced NH_4^+-N loading (higher HRT). At HRT of 10.7 h, the NH_4^+-N removal efficiency reached 99.9% but decreased to an average of 83% at an HRT of 2 h. This may be due to decreased reaction time and lower relative abundance of nitrifiers at lower HRT (Figure 3.5). Additionally, excessive growth of biomass may also decrease nitrogen removal efficiency (Hu et al., 2008).

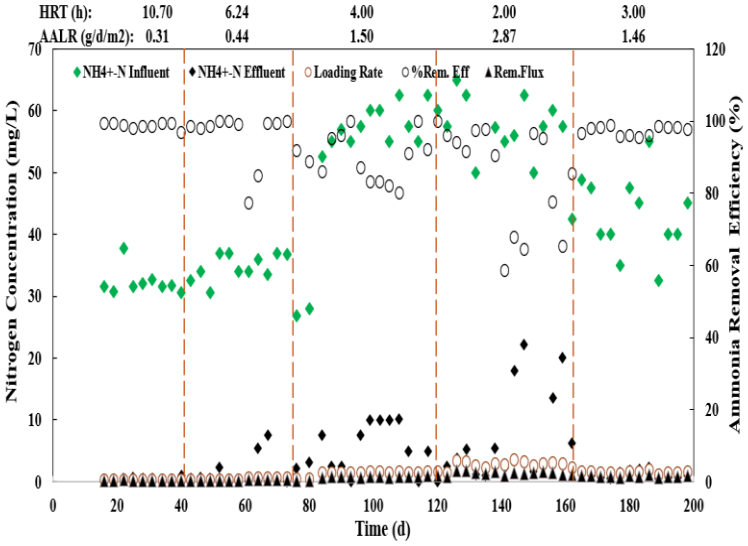
Table 3.3. Performance Comparison Between This Study and Other MABR Studies

		TCOD (mg/L)		$NH4^+$-N (mg/L)		TN	
Wastewater type	**HRT (h)**	**Inf. [a]**	**RE[b] (%)**	**Inf.**	**RE (%)**	**RE (%)**	**Reference**
Real MWW	12	-	-	57	80 – 92	8 – 55	Bunse et al., (2020)
Synthetic	12	250	90.2	50	96.4	83.7	Wu et al., (2019)
Synthetic	24	-	-	70	>76	>71.6	Lin et al, (2015)
Synthetic	12	348	86	77	94	84	Hu et al., (2008)
Synthetic	8	350	95	35	96	78.4	Dong et al, (2009)
-	8	-	88	-	95	-	Liu et al., (2007b)
Synthetic & Real PE	6.5	240	80	45	98	73.2	Semmens & Hanus, 1999
Real PE	3	275	98	55	96	67	This study

[a] *Inf – Influent,* [b] *RE – Removal Efficiency*

However, reactor performance became unstable at HRT of 2 h, therefore HRT was increased to 3h. The performance stabilized at HRT of 3 h and average NH_4^+-N removal efficiency increased 97%.

The high NH_4^+-N removal efficiency obtained in this study may be attributed to the ease of maintaining nitrifiers in biofilms (Downing & Nerenberg, 2008), in addition to the counter diffusion of oxygen and substrates in the biofilm which provided higher nitrification and denitrification activity in the system (Ravishankar et al., 2022).



HRT – hydraulic retention time,
AALR – average ammonia loading rate

Figure 3.3. Reactor performance dynamics at different stages of MABR operation. Influent and effluent NH_4^+-N concentrations, NH_4^+-N loading rates, NH_4^+-N removal flux, and the percentage of ammonia removal efficiency.

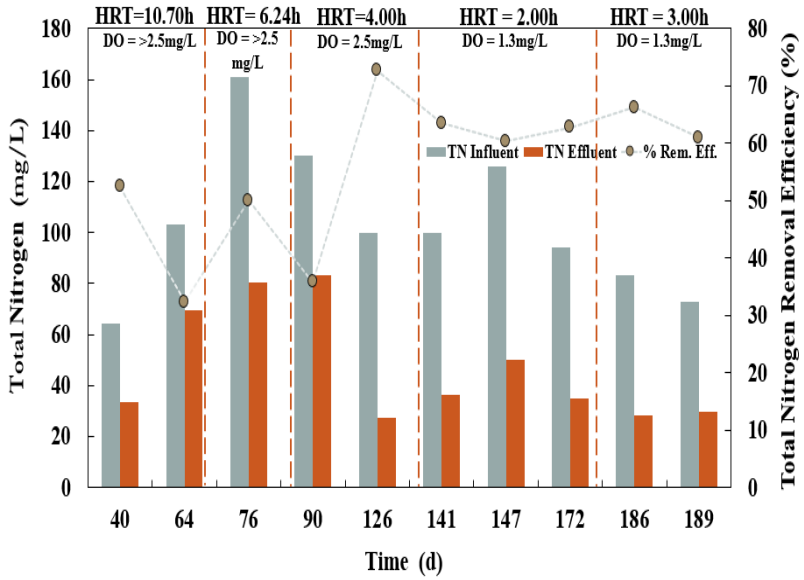


Figure 3.4. Total nitrogen (TN) dynamics and dissolved oxygen (DO) profile in the MABR at the different HRTs of operation.

3.3.3. Total nitrogen reduction and dissolved oxygen

The drop in bulk liquid dissolved oxygen (DO) over the course of the experiment (Figure 3. 4) likely arose from biofilm development on the membrane surface. The growth of biofilm increases mass transfer resistance near the membrane surface, leading to a decrease in gas flux (Shanahan & Semmens, 2005) as well as the increase in COD and nitrogen loading rates (Zielinska et al., 2012; Wei et al., 2012). In the first 90 days of the experiment, with HRTs of 10.7 h and 6.42 h, the DO in the bulk liquid was above 2.5 mg/L, dropping to 2.5 mg/L in stage 3 (HRT = 4 h). After over 120 days of operation, the DO in the bulk liquid of the reactor dropped further, to 1.3 mg/L, and remained stable over stages 4 and 5 (HRTs of 2 h and 3 h, respectively). A reduction in DO over an extended MABR operation was reported in Jang et al. (2002), where DO in the bulk solution dropped from 10 mg/L to 5 mg/L as the biofilm thickness increased from 200 μm to 300 μm.

A reduced DO concentration in the MABR coincides with a decrease in total nitrogen (TN). As shown in Figure 3.4, under higher HRT conditions (10.7 h and 6.24 h), the average TN removal was 45%. The low value could be attributed mainly to the complete aerobic oxidation of carbon, leaving an insufficient organic carbon to support denitrification. Additionally, aerobic conditions repress denitrifying enzymes (Sriwiriyarat et al., 2008; Hagedorn-Olsen, 1994). However, with the development of biofilm and a reduction in bulk liquid DO under HRT conditions of 4 h, 3 h, and 2 h, the TN removal increased. Hence, bulk DO concentration and biofilm thickness are critical parameters in MABR operations.

3.3.4. Biofilm microbial community structure

The bacterial community profiles of two biofilm and sludge samples obtained at different DO levels were determined by analyzing partial 16S sequence data. A total of 4614 OTUs with a minimum read-count of 2 were identified from 207884 SILVA annotated sequences. After removing all the singletons (low abundance species), 1214 OTUs were analyzed to compare the microbial

communities in the samples. Normalization of the sequences to the smallest sample (63590 sequences) showed that adequate sequencing depth was achieved (minimum sample coverage 98.1%). The alpha diversity index (Chao1) implied that more diverse microbial species were present at higher DO concentrations (the Chao1 indexes for SA2 and Z23 were 2328 and 3918, respectively). AOB and NOB were identified by searching the I table for the most reported genera: *Nitrobacter*, *Nitrospina*, *Nitrospira*, *Nitrotoga*, *Nitrolancea*, and *Nitrococcus*. The genera *Nitrospira* and *Nitrosomonas* were found in relatively higher abundance (1.6% to 2.9%, and 0.4% to 1.3%) in the samples. Nitrifying municipal wastewater treatment plants typically have *Nitrosomonas* RA in the range of 0.5-1.5% (Limpiyakorn, Shinohara et al. 2005). Appreciable quantity of Candidatus_*Nitrotoga* was also observed at HRT of 3h. *Candidatus_Nitrotoga* are dominant nitrite oxidizers under regular exposure to FNA and FA, with lower affinity to dissolved oxygen than *Nitrospira.* However, studies have demonstrated that they play significant role in biological nitrogen removal from mainstream wastewater (Zheng, Li et al. 2020). *Candidatus_Nitrotoga* has frequently been reported to be abundant over a wide temperature range (Kitzinger, Koch et al. 2018, Wegen, Nowka and Spieck 2019).

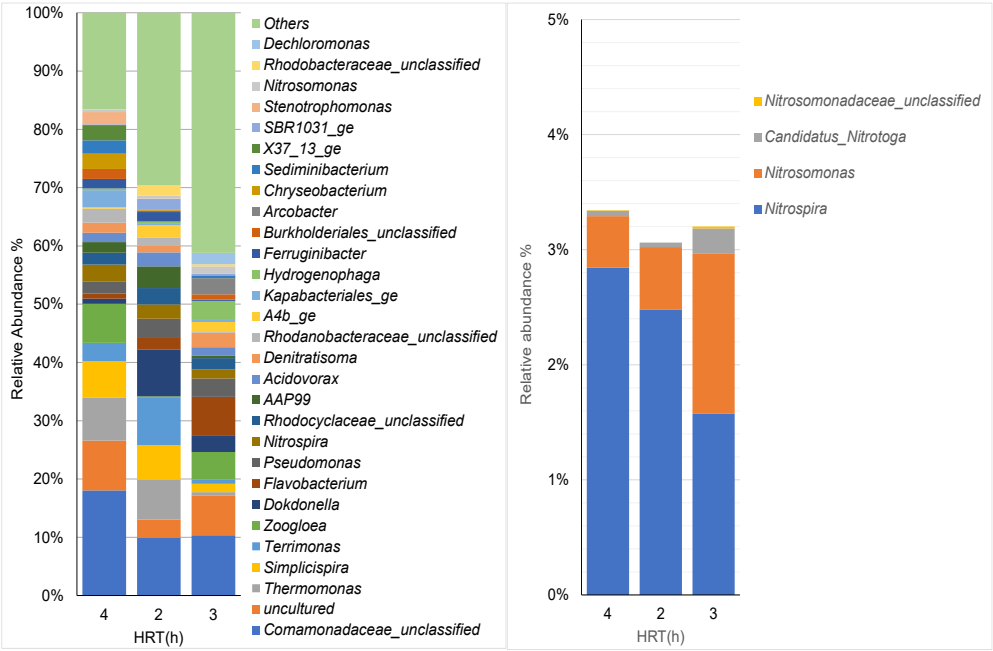


Figure 3.5. Relative abundance (%) in the MABR of the 20 most dominating genera (left) and the relative abundance (%) of AOB and NOB (right). Genera are color coded. The X-axis indicates the HRT (h).

3.3.5. Microbial community analysis

Microbial analysis indicated that the bacterial community dynamics correlated strongly with the HRT and DO levels. AOBs (*Nitrosomonas* and *Nitrosomonadaceae*) (Lin et al., 2015, Zhang et al., 2011) were present. However, while *Nitrosomonas* clusters were prominent, *Nitrosomonadaceae* were low at all HRTs studied. Nitrosomonadaceae are known to contain two genera *Nitrosomonas* and *Nitrosospira* (Prosser, Head and Stein 2014), both of which have been reported as dominant AOBs in low ammonia environments (Limpiyakorn, Shinohara et al. 2005, Kurisu, Kurisu et al. 2007). The relative abundance of *Nitrosomonas* increased with high surface loading rates (lower HRT), which shows their capability to adapt to continuously increasing contaminated environment (Figure 3.5). *Nitrosomonas* reportedly has capability for high nitrification rate under different operation conditions (Canto-Encalada, Tec-Campos et al. 2022).

NOBs present within the reactor were *Nitrospira* and *Nitrotoga*. The relative abundance of *Nitrospira* consistently declined as HRT was decreased from 4 h to 2 h. This may have resulted from the constant high and variables loading of the reactor from raw wastewater and competition for DO with heterotrophs and *Nitrosomonas* both of which possess faster growth rates and higher affinity for DO (Okabe, Aoi et al. 2011). *Nitrotoga* on the other hand, had relatively much lower RA than *Nitrospira* at all HRTs, which suggests that it is not a dominant nitrite oxidizer in this MABR. *Nitrospira* is often the most predominant nitrite oxidizer in municipal wastewater treatment plants. At HRT of 3h, the RA of *Nitrosomonas* and *Nitrospira* appeared to be at equilibrium, which signifies the system had attained and maintained steady state. The combined activities of these bacteria within the MABR resulted in high ammonia removal efficiency over the extended operation period.

Another genus with substantive relative abundance at all HRTs was *Flavobacterium* a strictly aerobic bacterium that utilizes amino acids as an energy source *Flavobacterium* is capable of degrading organics and maintaining the microbial community structure through the secretion of extracellular polymers (Aslam et al., 2005). Aerobic denitrifying bacteria (ADB), including *Pseudomonas*, *Zoogloea*, *Comamonas*, and *Dechloromonas*, responsible for aerobic denitrification and COD removal (Zhou et al., 2020; Lin et al., 2015), were also found within the system. Anaerobic denitrifying bacteria includes; *Denitratisoma*, *Thermomonas, Simpliscispira* and *Acidovorax* with denitrification potentials (Zhou, Li et al. 2020, Zhuang, Wu et al. 2020) were also in the MABR. The coexistence of NOB, AOB, denitrifiers (ADB &AnDB) and other bacteria were responsible for the simultaneous nitrification, denitrification, and the removal of organics that occurred within the MABR.

In general, the microbial community populations identified in each stage of this study were similar, all major municipal wastewater microbes were present at all the HRTs, but differed in

relative abundance, which may have resulted due to the difficulty in retaining slower growing microorganisms with short HRT (Koch, Lücker et al. 2015). Thus, HRT impacted the microbial community structure but not to the extent of performance inhibition.

3.4. Energy evaluation for pollutant removal in MABR

The energy requirements for pollutants (COD and NH_4^+-N) removal in MABR operation correlates with influent characteristics, membrane surface area, oxygen transfer rate (OTR), oxygen transfer efficiency (OTE) of the aeration system and the overall efficiency of the system. Evaluation of this energy involves a two-step calculation.

a) Determination of air/oxygen requirement

b) Estimation of the power required for the air/oxygen supply

3.4.1. Determination of air/oxygen requirement

Oxygen needed for complete oxidation of COD and NH_4^+-N ($O_{2,T}$) (g/d) = oxygen needed for each pollutant oxidation (mg/L) multiplied by daily flowrate (Q_{df}) (m^3/d).

$(O_{2,T})$ (g/d) = O_2 $(C_{COD,in} + C_{NH4+-N,in} * 4.57)$ (mg/L) * (Q_{df}) (m^3/d) …… (3)

- 1g of O_2 is required for complete oxidation of 1gCOD, while 4.57g of O_2 are required for the complete oxidation of 1g of NH_4^+-N to NO_3-N (complete nitrification).

Membrane surface needed to supply the oxygen can be determined from equation 4

MSA (m^2) = $\frac{O_{2,T}}{OTR}$ ……..... (4)

Air flowrate (g/d) = $\frac{\text{Oxygen needed for each pollutant oxidation } (O_{2,T})}{\text{Oxygen transfer efficiency (OTE)}}$ ……..... (5)

Oxygen needed (m^3/d) = $\frac{O_{2,T}}{\rho O_2}$ ……. (6)

ρO_2 = density of oxygen (g/m^3)

3.4.2. Estimation of the power required for the air/oxygen supply

General formular for power calculation is given by equation 7 (Metcalf and Eddy, 2014).

$$P_w = \frac{wRT}{28.97_{ne}}\left[\left(\frac{p_2}{p_1}\right)^n - 1\right] \quad \text{..... (7)}$$

Where:

P_w = Power requirement for blower (kW)
w = weight of air flow rate, kg/s
R = universal gas constant, 8.314 kJ/kmol. K
T = absolute inlet temperature, K
P_1 *= absolute inlet pressure, atm.*
P_2 *= absolute outlet pressure, atm.*
n = (γ-1)/γ, where γ is specific heat ratio. For dry air, γ = 1.4
M = molecular weight of dry air (28.97kg/kmol)
e = efficiency (0.70 - 0.90 for compressors)
vol = vol. of wastewater treated per day

Equation 8 can be simplified using the isentropic relationship between temperature and pressure (Perry, 1950) to give equation 10.

$$\frac{T_2}{T_1} = \left(\frac{P_2}{P_1}\right)^n \quad \text{..... (8)}$$

$$\frac{C_p}{C_v} = \gamma \quad \text{..... (9)}$$

$$P = 8.314n\left(\frac{T_2 - T_1}{eM}\right)w \quad \text{..... (10)}$$

Equation 11 is in tonnes per hour (t/h) (Perry,1950). Converting to kg/s gives equation 12.

$$P = 8.314n^{-1}\left(\frac{T_2 - T_1}{eM}\right)\frac{w * 1000}{3600} \quad \text{..... (11)}$$

$$P = 2.31n^{-1}\left(\frac{T_2 - T_1}{eM}\right)w \quad \text{..... (12)}$$

Table 3.4. Operating Condition for Aeration Energy Estimation

Parameter	Unit	Value
COD	mg/L	275
NH_4^+-N	mg/L	55
Daily flowrate	L/day	0.68
Oxygen density	Kg/m^3	1.43
Oxygen transfer efficiency	%	60
Oxygen transfer rate[a]	g O_2/m^2.day	4.157
Reference temperature	K	273.15
Operating temperature	K	304.5
Reference pressure	pa	101325
Operating pressure	pa	13789.5
Pump efficiency (η)[b]	%	70
Oxygen concentration in air	%	21

a = Zhou et al.,2020; b. = assuming a compressor efficiency of 0.7 (Metcalf, Eddy et al., 2014).

Table 3.5. Pump Power Calculation

Parameter	Unit	Calculated Value
COD (Load)	mg/day	187
NH_4^+-N (Load)	mg/day	171
Total oxygen required ($O_{2,T}$)	mg/day	358
Membrane surface area (MSA)	cm^2	900
Vol. of oxygen required	mL/day	256
Oxygen supplied	mL/day	426
Air supplied	mL/day	2029
Air supplied	mL/hr	85
Power requirement	kW	$4.732 * 10^{-7}$
Energy	kWh/ m^3	0.02

3.4.3. Energy evaluation

Energy supplied by the pump can be calculated using the energy- power relationship.

$$\text{Energy (kWh/ m}^3\text{)} = \frac{\text{Power (kW)} * \text{Time (h)}}{\text{Vol (m}^3\text{)}} \quad \text{..... (13)}$$

Aeration energy requirement analysis shows that the MABR can be operated with very low aeration energy of 0.02 kWh/m^3.

3.5. Conclusion

The pollutant removal capacity of an MABR treating real municipal wastewater was investigated under different HRTs (10.7 h, 6.24 h, 4 h, 3 h, and 2 h). The TCOD removal efficiency increased from 90% to 99% as the HRT was gradually reduced from 10.7 h to 4 h. An increase in the organic surface loading, through HRT reduction, promoted the growth of heterotrophic bacteria responsible for organic matter consumption, thereby enhancing TCOD removal. However, this performance could not be sustained at certain operational condition. A reduction of the HRT to 2 h led to a 9% reduction in TCOD removal efficiency and to process instability. A similar trend was observed for $NH4^+$-N reduction. When the system surface load was lessened by increasing the HRT to 3h, a swift recovery was observed along with an 8% increase in TCOD removal efficiency. Although the best MABR performance was achieved at an HRT of 4 h, the decrease in TCOD removal efficiency between HRTs of 4 h and 3 h was statistically insignificant, indicating that the shorter HRT produced competitive results. Additionally, under varying hydraulic loads, the MABR maintained sufficient biodiversity, richness and adequate microbial density to sustain the reactor performance for more than 200 days. MABRs operated at lower HRTs are considered to be economically more favourable than MABRs operated at higher HRTs (Aybar et al., 2014b, Grosser, 2017).

Despite constant fluctuations in influent composition from the use of real municipal wastewater PE applied in this study; results indicated acceptable effluent quality under a short HRT of 3 h and low aeration energy. The maintenance of a short HRT reduces the area and volume requirements in an MABR, thus improving the energy efficiency, profitability and sustainability in wastewater treatment plants.

3.6. References

Adams, V.D., 2017. *Water and wastewater examination manual*. Routledge.

Aslam, Z., Im, W.T., Kim, M.K. and Lee, S.T., 2005. Flavobacterium granuli sp. nov., isolated from granules used in a wastewater treatment plant. *International journal of systematic and evolutionary microbiology*, *55*(2), pp.747-751.

Aybar, M., Pizarro, G., Boltz, J.P., Downing, L. and Nerenberg, R., 2014. Energy-efficient wastewater treatment via the air-based, hybrid membrane biofilm reactor (hybrid MfBR). *Water science and technology*, *69*(8), pp.1735-1741.

Bui, X.T., Chiemchaisri, C., Fujioka, T. and Varjani, S. eds., 2019. *Water and wastewater treatment technologies*. Springer Singapore.

Canto-Encalada, G., D. Tec-Campos, J. D. Tibocha-Bonilla, K. Zengler, A. Zepeda and C. Zuñiga (2022“. "Flux balance analysis of the ammonia-oxidizing bacterium Nitrosomonas europaea ATCC19718 unravels specific metabolic activities while degrading toxic compoun”s." PLoS Computational Biology **18**(2): e1009828.

Casey, E., Glennon, B. and Hamer, G., 1999. Oxygen mass transfer characteristics in a membrane aerated biofilm reactor. *Biotechnology and bioengineering*, *62*(2), pp.183-192.

Casey, E., Syron, E., Shanahan, J.W. and Semmens, M.J., 2008. Comparative economic analysis of full scale MABR configurations. In *2008 IWA North American Membrane Research Conference, University of Massachusetts, Amherst, USA, August 10-13, 2008*.

Castrillo, M., Díez-Montero, R., Esteban-García, A.L. and Tejero, I., 2019. Mass transfer enhancement and improved nitrification in MABR through specific membrane configuration. *Water research*, *152*, pp.1-11.

Chen, R.D., Semmens, M.J. and LaPara, T.M., 2008. Biological treatment of a synthetic space

mission wastewater using a membrane-aerated, membrane-coupled bioreactor (M2BR). *Journal of Industrial Microbiology and Biotechnology*, *35*(6), pp.465-473.

Cole, A.C., Shanahan, J.W., Semmens, M.J. and LaPara, T.M., 2002. Preliminary studies on the microbial community structure of membrane-aerated biofilms treating municipal wastewater. *Desalination*, *146*(1-3), pp.421-426.

Dong, W.Y., Wang, H.J., Li, W.G., Ying, W.C., Gan, G.H. and Yang, Y., 2009. Effect of DO on simultaneous removal of carbon and nitrogen by a membrane aeration/filtration combined bioreactor. *Journal of Membrane Science*, *344*(1-2), pp.219-224.

Downing, L.S. and Nerenberg, R., 2008. Effect of bulk liquid BOD concentration on activity and microbial community structure of a nitrifying, membrane-aerated biofilm. *Applied microbiology and biotechnology*, *81*(1), pp.153-162.

Grosser, A., 2017. The influence of decreased hydraulic retention time on the performance and stability of co-digestion of sewage sludge with grease trap sludge and organic fraction of municipal waste. *Journal of environmental management*, *203*, pp.1143-1157.

Hagedorn-Olsen, C., Møller, I.H., Tøttrup, H. and Harremoës, P., 1994. Oxygen reduces denitrification in biofilm reactors. *Water Science and Technology*, *29*(10-11), p.83.

He, H., B. M. Wagner, A. L. Carlson, C. Yang and G. T. Daigger 202“. "Recent progress using membrane aerated biofilm reactors for wastewater treatme”t." *Water Science and Technology, 84*(9), pp. 2131-2157.

Heffernan, B., Shrivastava, A., Toniolo, D., Semmens, M. and 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), pp.285-297.

Hibiya, K., Terada, A., Tsuneda, S. and Hirata, A., 2003. Simultaneous nitrification and denitrification by controlling vertical and horizontal microenvironment in a membrane-aerated

biofilm reactor. *Journal of biotechnology*, *100*(1), pp.23-32.

Houweling, D., Peeters, J., Cote, P., Long, Z. and Adams, N., 2017. Proving membrane aerated biofilm reactor (MABR) performance and reliability: results from four pilots and a full-scale plant. *Proceedings of the Water Environment Federation*, *2017*(16), pp.272-284.

Kitzinger, K., Koch, H., Lücker, S., Sedlacek, C.J., Herbold, C., Schwarz, J., Daebeler, A., Mueller, A.J., Lukumbuzya, M., Romano, S. and Leisch, N., 2018. Characterization of the first "Candidatus Nitrotoga" isolate reveals metabolic versatility and separate evolution of widespread nitrite-oxidizing bacteria. *MBio*, *9*(4), pp.e01186-18.

Koch, H., S. Lücker, M. Albertsen, K. Kitzinger, C. Herbold, E. Spieck, P. H. Nielsen, M. Wagner and H. Daims (2015". "Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus Nitrospi"a." Proceedings of the national academy of sciences **112**(36): 11371-11376.

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

Kurisu, F., F. Kurisu, Y. Sakamoto and O. Yagi (2007". "Effects of ammonium and nitrite on communities and populations of ammonia-oxidizing bacteria in laboratory-scale continuous-flow reacto"s." FEMS microbiology ecology **60**(3): 501-512.

Lema, J.M. and Martinez, S.S. eds., 2017. *Innovative wastewater treatment & resource recovery technologies: impacts on energy, economy and environment*. IWA publishing.

Li, J., Zhu, L.P., Xu, Y.Y. and Zhu, B.K., 2010. Oxygen transfer characteristics of hydrophilic treated polypropylene hollow fiber membranes for bubbleless aeration. *Journal of Membrane Science*, *362*(1-2), pp.47-57.

Li, M., Du, C., Liu, J., et al., 2018. Mathematical modeling on the nitrogen removal inside the membrane-aerated biofilm dominated by ammonia-oxidizing archaea (AOA): effects of

temperature, aeration pressure and COD/N ratio. *Chemical Engineering Journal*, *338*, pp.680-687.

Li, Y.Z., He, Y.L., Ohandja, D.G., et al., 2008. Simultaneous nitrification–denitrification achieved by an innovative internal-loop airlift MBR: Comparative study. *Bioresource Technology*, *99*(13), pp.5867-5872.

Li, Y. and Zhang, K., 2018. Pilot scale treatment of polluted surface waters using membrane-aerated biofilm reactor (MABR). *Biotechnology & Biotechnological Equipment*, *32*(2), pp.376-386.

Limpiyakorn, T., Y. Shinohara, F. Kurisu and O. Yagi (2005“. "Communities of ammonia-oxidizing bacteria in activated sludge of various sewage treatment plants in Tok”o." FEMS microbiology ecology **54**(2): 205-217.

Lin, J., Zhang, P., Yin, J., Zhao, X. and Li, J., 2015. Nitrogen removal performances of a polyvinylidene fluoride membrane-aerated biofilm reactor. *International Biodeterioration & Biodegradation*, *102*, pp.49-55.

Martin, K.J., Boltz, J.P. and Nerenberg, R., 2012, January. The Membrane Biofilm Reactor (MBfR) for Wastewater Treatment: Applications, Design Considerations, and Technology Outlook. In *Proceedings of 85th Annual Water Environment Federation Technical Exposition & Conference* (pp. 4032-4044).

Mei, X., Guo, Z., Liu, J., et al., 2019. Treatment of formaldehyde wastewater by a membrane-aerated biofilm reactor (MABR): the degradation of formaldehyde in the presence of the co-substrate methanol. *Chemical Engineering Journal*, *372*, pp.673-683.

Metcalf, Eddy, M. Abu-Orf, G. Bowden, F. L. Burton, W. Pfrang, H. D. Stensel, G. Tchobanoglous, R. Tsuchihashi and AECOM (2014). Wastewater engineering: treatment and resource recovery, McGraw Hill Education.

Perez-Calleja, P., Aybar, M., Picioreanu, C., et al., 2017. Periodic venting of MABR lumen allows high removal rates and high gas-transfer efficiencies. *Water research*, *121*, pp.349-360.

Perry, J. H. (1950). Chemical engineers' handbook, ACS Publications.

Ravishankar, H., A. Nemeth, G. Massons, D. Puig, D. Zardoya, N. Carpi, P. N. Lens and B. Heffernan (2022“. "Factors impacting simultaneous nitrification and denitrification in a membrane aerated biofilm reactor (MABR) system treating municipal wastewat”r." *Journal of Environmental Chemical Engineering 10*(5), pp.108120.

Semmens, M. and Hanus, D., 1999. Studies of a membrane aerated bioreactor for wastewater treatment. *Membrane Technology*, *1999*(111), pp.9-13.

Sevanthi, R., Salehi, M., Jackson, A., Morse, A. and Callahan, M.R., 2019, July. Long term biological treatment of space habitation waste waters in a one stage MABR: comparison of operation for N and C oxidation with and without simultaneous denitrification. In *International Conference on Environmental Systems (ICES)*.

Shanahan, J.W. and Semmens, M.J., 2004. Multipopulation model of membrane-aerated biofilms. *Environmental science & technology*, *38*(11), pp.3176-3183.

Shanahan, J.W. and Semmens, M.J., 2015. Alkalinity and pH effects on nitrification in a membrane aerated bioreactor: an experimental and model analysis. *Water Research*, *74*, pp.10-22.

Sriwiriyarat, T., Ungkurarate, W., Fongsatitkul, P. and Chinwetkitvanich, S., 2008. Effects of dissolved oxygen on biological nitrogen removal in integrated fixed film activated sludge (IFAS) wastewater treatment process. *Journal of Environmental Science and Health, Part A*, *43*(5), pp.518-527.

Syron, E. and Casey, E., 2008. Membrane-aerated biofilms for high rate biotreatment: performance appraisal, engineering principles, scale-up, and development requirements. *Environmental science & technology*, *42*(6), pp.1833-1844.

Terada, A., Hibiya, K., Nagai, J., Tsuneda, S. and Hirata, A., 2003. Nitrogen removal characteristics and biofilm analysis of a membrane-aerated biofilm reactor applicable to high-strength nitrogenous wastewater treatment. *Journal of bioscience and bioengineering*, *95*(2), pp.170-178.

Tian, H., Xu, X., Qu, J., et al., 2020. Biodegradation of phenolic compounds in high saline wastewater by biofilms adhering on aerated membranes. *Journal of hazardous materials*, *392*, p.122463.

Tian, H., Zhang, H., Li, P., et al., 2015. Treatment of pharmaceutical wastewater for reuse by coupled membrane-aerated biofilm reactor (MABR) system. *RSC advances*, *5*(85), pp.69829-69838.

Okabe, S., Y. Aoi, H. Satoh and Y. Suwa (2011“. "Nitrification in wastewater treatme”t." Nitrification: 405-433.

Parker, D.S., 1975. Process Design Manual for Nitrogen Control.

Wang, L., Pang, Q., Peng, F., et al., 2020. Response characteristics of nitrifying bacteria and archaea community involved in nitrogen removal and bioelectricity generation in integrated tidal flow constructed wetland-microbial fuel cell. *Frontiers in microbiology*, *11*, p.1385.

Wegen, S., Nowka, B. and Spieck, E., 2019. Low temperature and neutral pH define “Candidatus Nitrotoga sp.” as a competitive nitrite oxidizer in coculture with Nitrospira defluvii. *Applied and environmental microbiology*, *85*(9), pp.e02569-18.

Wei, X., Li, B., Zhao, S., et al., 2012. COD and nitrogen removal in facilitated transfer membrane-aerated biofilm reactor (FT-MABR). *Journal of Membrane Science*, *389*, pp.257-264.

Zhang, J., Wu, P., Hao, B. and Yu, Z., 2011. Heterotrophic nitrification and aerobic denitrification by the bacterium Pseudomonas stutzeri YZN-001. *Bioresource technology*, *102*(21), pp.9866-9869.

Zhuang, H., Z. Wu, L. Xu, S.-Y. Leu and P.-H. Lee (2020“. "Energy-efficient single-stage nitrite shunt denitrification with saline sewage through concise dissolved oxygen (DO) supply: process performance and microbial communiti”s." Microorganisms **8**(6): 919.

Zhou, H., 2013. *Tertiary Nitrification Using Membrane Aerated Biofilm Reactors: Process Optimization, Characterization and Model Development* (Doctoral dissertation).

Zhou, Y., Li, R., Guo, B., et al., 2020. Greywater treatment using an oxygen-based membrane biofilm reactor: Formation of dynamic multifunctional biofilm for organics and nitrogen removal. *Chemical Engineering Journal*, *386*, p.123989.

Zielinska, M., Bernat, K., Cydzik-Kwiatkowska, A., Sobolewska, J. and Wojnowska-Baryla, I., 2012. Nitrogen removal from wastewater and bacterial diversity in activated sludge at different COD/N ratios and dissolved oxygen concentrations. *Journal of environmental sciences*, *24*(6), pp.990-998.

Chapter 4 – Structural and Microbial Dynamics Analyses of MABR Biofilms

4.1. Introduction

The direct release of untreated or poorly treated MWW into water bodies contributes more than 50% of the total nonpoint nutrient load to the aquatic ecosystem (Rout, Shahid et al. 2021). Water bodies generally have low threshold nutrient content, and a small increase in nutrient load can alter the structure and function of the different life forms in that ecosystem (Dodds and Welch 2000). Thus, eutrophication control through nutrient removal from wastewater is a global research priority. Conventional activated sludge (CAS) which is the most commonly used technology for MWW treatment, although highly effective, has very low energy efficiency (McCarty, Bae and Kim 2011), therefore, the development of an alternative technology with higher energy efficiency and the ability to comply with high effluent discharge quality will represent a significant milestone in the treatment of MWW. The MABR is a well-researched biofilm technology with proven potentials to meet the aforementioned conditions (Côté, Peeters et al. 2015, Heffernan, Shrivastava et al. 2017, Peeters, Long et al. 2017).

MABR biofilm properties control all key performance indices in MABR operations (Martin and Nerenberg 2012, Sanchez-Huerta, Fortunato et al. 2022) while regional stratification within the biofilm is controlled by substrates and oxygen loading rates (LaPara, Cole et al. 2006, He, Wagner et al. 2021). [2] Under high surface loading rates, MABR biofilm growth rate can exceed the erosion (small-particle removal) rate, resulting in a thick biofilm and its attendant challenges (Picioreanu,

[2] A version of this chapter has been published in Journal of Environmental Engineering, 2023 as: S Ukaigwe, Y Zhang, K Lee, Y Liu "Structural and microbial dynamics analyses of MABR biofilms." *Journal of Environmental Engineering,* 2023

Van Loosdrecht and Heijnen 2001). Strategies that have been applied to tackle biofilm overgrowth includes; intermittent air sparging, increased recirculation flow velocity, combination of membrane washing and air scouring (Terada, Hibiya et al. 2003, Côté, Peeters et al. 2015, Syron, Semmens and Casey 2015, Da Silva, Matsumoto et al. 2018, Bunse, Orschler et al. 2020), but most strategies result in extended process perturbation (Semmens, Dahm et al. 2003, Syron and Casey 2008, Bunse, Orschler et al. 2020, He, Wagner et al. 2021, Wagner, Daigger and Love 2022). In this study, membrane washing, initialized by a drop in bulk DO to a set-point of 0.2 mg/L, was exclusively applied as the biofilm thickness control mechanism. This operational procedure resulted in reduced process upsets, while high and stable performance was maintained over extended operational periods. Operational procedure involving; intermittent membrane cleaning, biomass recirculation, and progressive surface loading rates was used to study performance stability, pollutant removal efficiency, and bacterial community succession of an MABR treating medium-strength municipal wastewater.

4.2. Materials and methods

4.2.1. Influent and inoculum sludge properties

The medium strength synthetic wastewaters used in this study had the following composition: NaOAc 938 mg/L, $C_3H_5NaO_2$ 210 mg/L, NH_4Cl 191mg/L, K_2HPO_4 30 mg/L, KH_2PO_4 25 mg/L, $CaCl_2.2H_2O$ 15 mg/L, $MgSO_4.7H_2O$ 12.5 mg/L, $FeSO_4.7H_2O$ 10 mg/L, 0.5 mL/L micronutrient solution, as described in Tay, Liu et al., (2002) and Syron, Semmens et al., (2015). The micronutrient solution contained: H_3BO_4 0.05 g/L, $ZnCl_2$ 0.05 g/L, $CuCl_2$ 0.03 g/L, $MnSO_4.H_2O$ 0.05 g/L, $(NH_4)_6$ Mo_7O_{24} $4H_2O$ 0.05 g/L, $AlCl_3$ 0.05 g/L, $CoCl_2$ $6H_2O$ 0.05 g/L, $NiCl_2$ 0.05 g/L (Tay, Liu and Liu 2002). As a result, the average COD and ammonia concentrations were 455 ± 30 mg/L and 37 ± 9 mg/L respectively for the entire study period. Prepared influent was stored at 4 °C and used within 1 week.

4.2.2. Experimental procedure

This study was performed over a period of ~6 months. The start-up took about 30 days, however additional 14 days was used for process parameter adjustments (gas pressure and aeration control adjustments) and process stabilization. Subsequently, a 140-day continuous operation was conducted based on the parameters determined from the initial operation conditions. The MABR was operated at HRT of 48h in the first 42 days of operation and sequentially reduced until 2.5h over a period of 185 days as the system stabilized per stage. The entire operation was done in 6 consecutive stages with synthetic medium-strength MWW at room temperature (22 ± 0.3°C) and no pH control. Each HRT represented a stage, and each stage was operated until steady-state is attained (at least three weeks) using the operating conditions outlined in Table 4.1. A steady-state was considered to have been reached when pollutant (COD and NH4+-N) removal efficiencies were within a 10% variation consecutively over two to three readings (4 – 6 days). Returned activated sludge obtained from a full-scale biological nutrient removal wastewater treatment plant in Alberta was added to the reactor as inoculum. Influent flow and air supply were continuous at all operation conditions. The reactor unit was maintained in a batch mode for 14 days after sludge inoculation to ensure there was sufficient biofilm formation to initiate the process.

Membrane biofilm thickness was controlled by an intermittent washing of the membrane with clean water for about 60 seconds at a flowrate of 250 – 500 mL/min. To wash the membranes, the influent line is transferred into the wash water tank and the flow rate adjusted. The feed tube is returned to the influent tank afterwards. Membrane wash was employed whenever the dissolved oxygen (DO) dropped to 0.2 mg/L. Washing was done severally at the start-up stage before stability was attained, and on days; 56, 84, and 110 after the stabilization periods. The bulk liquid DO was continuously monitored and was maintained between 0.2 – 0.6 mg/L.

4.2.3. Biofilm

4.2.3.1. Live/dead biofilm assay

Biofilm samples were cut from the MABR, stained with 1 mg/mL propidium iodide solution (LIVE/DEAD Backlight Bacterial Viability Kits) using 100 µL SYTO9 (Molecular Probes), and incubated in the dark at room temperature for 20 min. The biofilm samples were washed with phosphate buffered saline (PBS) after staining. The distribution of viable and nonviable cells was observed in the biofilm using fluorescence microscopy (Leica DM750 Fluorescence Microscope, PA, USA) at wavelengths of 488 nm and 515 nm, which correspond, respectively, to the excitation wavelengths of the SYTO9 and the propidium iodide contained in the staining reagent.

Table 4.1. Operating Parameters for Each Stage of the Study

Stage	Time (d)	HRT[a] (h)	TCOD[b] Loading rate (g/m^2 - d)	NH_4^+-N[c] Loading rate (g/m^2 - d)	Lumen Press (kPa)
Start up	1-26	48	0.4 ± 0.1	0.03 ±0.001	1–8 - 2.2
1	27- 42	24	0.81± 0.1	0.07 ± 0.005	2
2	44 -76	10	2.01 ± 0.3	0.14 ± 0.05	2
3	80 -108	6	3. 0 ± 0.5	0.16 ± 0.04	2
4	110 -145	4	5.7 ± 0.6	0.57± 0.06	2
5	145 -180	2.5	10.0 ± 0.7	0.93 ± 0.07	2

[a] hydraulic retention time, [b] total chemical oxygen demand, [c] ammonium-nitrogen, [d] ± standard deviations of the average parameter values

4.2.3.2. MABR biofilm density and thickness measurements

To obtain the biofilm areal density, two representative membranes with biofilm were detached from the membrane bundle and heated overnight in an oven at 105 ºC. The dried samples

were weighed, the biofilm was mechanically removed from the membrane as described by Hu et al., (2013) and the membranes were reweighed. Biofilm density was defined as the difference in weight between the membrane with biofilm and the membrane without biofilm, with the dry biomass normalized to the growth area of the wet biofilm for the calculation of biomass per unit area of membrane. Biofilm mass was calculated based on the total number of membranes in the reactor. Biofilm thickness was measured using a digital Vernier caliper.

4.2.3.3. EPS component extraction from biofilm

To extract extracellular polymeric substances (EPS) from the biofilm, one membrane with a length of approximately 200 mm was cut off the reactor membrane bundle and the biofilm on the membrane surfaces was removed using the method described by (Luo, Chen et al. 2015). The detached biofilms were centrifuged at 3000 g (Thermo Sorvall Lynx 4000, Centrifuge, USA) at 21± 0.3 ˚C) for 5 min and the supernatant was removed. Approximately half of the biofilm sludge was used to determine the volatile suspended solids (VSS). VSS were measured according to standard methods (APHA AWWA 1998).

The remaining half of the biofilm sludge was mixed with 20 mL phosphate buffered saline (PBS), followed by 15 min of vortex mixing (VWR® Vortex Mixer, Fixed Cup Head, Avantor, USA) at room temperature. The mixture was treated by ultrasound (FS30H Ultrasonic cleaner, Fisher Scientific, PA, Mexico) for 3 min and incubated in a water bath (Elmasonic E30H, Germany) at 80 ˚C for 20 min. The treated biofilm sludge was centrifuged for 15 min, at 10,000 g (Thermo Sorvall Lynx 4000, Centrifuge, USA) and the supernatant was filtered using 0.45 μm membrane filters. Filtrate containing EPS was purified in a dialysis membrane (Spectrum™ Labs Spectra/Por™) for 24 h. Polysaccharide and protein contents of the filtrate were determined using the phenol-sulfuric acid method with glucose as the standard (Frølund, Palmgren et al. 1996) and

the Coomassie brilliant blue G-250 dye-binding method using bovine serum albumin as the standard (Pierce and Suelter 1977).

4.2.3.4. Deoxyribonucleic acid (DNA) extraction

DNA was extracted from the reactor inocula and from biofilm samples collected at HRTs of 24, 10, 6, 4, and 2.5 h, respectively, using a Dneasy PowerSoil Kit (Qiagen Inc., Toronto, Canada) and following the manufacturer's protocol. The purity and concentration of the DNA extracted from each sample were measured with NanoDrop One (ThermoFisher, Waltham, MA). DNA amplicon samples were stored at -20 °C, before being sent to the Génome Québec Innovation Centre (Montréal, QC, Canada) for barcoding and sequencing on the Illumina MiSeq PE250 platform using the primer pair 515F/806R. Forward and reverse reads of the raw sequence were paired and screened, and chimeras were removed with the "DADA2" algorithm in the QIIME2 pipeline (Callahan, McMurdie et al. 2016). Taxonomy was determined with 99% similarity in the GreenGenes database, version 13_8 (McDonald, Price et al. 2012, Werner, Koren et al. 2012)

4.3. Results and discussion

4.3.1. Reactor performance

4.3.1.1. COD removal

The reactor maintained a high organic carbon removal efficiency at all HRTs for the duration of this experiment (Figure 4.1a), which may have resulted from both the high performance of the MABR and the ease of sodium acetate biodegradation by heterotrophic and aerobic microorganisms. Similar observations have been reported previously for MABR treatments (Da Silva, Matsumoto et al. 2018, Houweling and Daigger 2019). For the first 44 days, with surface loading rates of 0.37 $gCOD/m^2/d$ and 0.84 $gCOD/m^2/d$ (corresponding to HRTs of 48 h and 24 h), the average COD removal efficiencies were ~ 73% and ~ 89%, respectively. The COD removal efficiency progressively increased to 94% and 98% after 60 days (day 45 to day 105) when surface loading

rates (SLRs) were further increased to 2.1 and to 3.1 gCOD/m^2/d (HRT: 10-6 h), as biofilm rapidly developed on the membrane. The membrane surfaces were non-aggressively cleaned to prevent excessive biomass build-up and to maintain biofilm stability. Over the next 40 days (day 145), at a higher loading of 5.81 gCOD/m^2/d (HRT: 4 h), the reactor performance declined by 6% to an average removal efficiency of ~ 92%; this might have been due to the increased load and the biofilm loss from cleaning. However, the 92% average organic carbon removal efficiency was sustained over the next 70 days (day 185) despite an additional load increase to an average of 10.5 gCOD/m^2/d (HRT: 2.5 h); no further membrane cleaning was applied and performance stability was sustained. Although each membrane cleaning period was preceded by a drop in performance, the reactor performance returned to pre-cleaning values within 6-8 days (Figure 4.1a). The speedy performance recovery could have been due to the gentle membrane cleaning strategy used; such benign removal of loose biofilm minimally impacts microbial community activities. Bunse et al., (2020) and other researchers have reported a performance lag of several months after membrane cleaning (Bunse, Orschler et al. 2020, He, Wagner et al. 2021).

4.3.1.2. Ammonium-nitrogen removal

For the first 25 days of operation, at an average SLR of 0.03 gN/m^2/d (HRT: 48 h) and average influent concentration of 25 mg/L, the NH_4^+-N removal efficiency averaged 72% (Figure 4.1b). After 80 days (day 105) of MABR operation, and a 10-fold increase in the system load from 0.03 to 0.3 gN/m^2/d through a step-wise decrease in HRT from 24 h to 6 h, an increase in average influent NH_4^+-N concentration to 36 mg/L, and gentle membrane cleaning, the overall system moved toward stability, providing conditions that nitrifiers require to thrive. The NH_4^+-N removal efficiency improved correspondingly and eventually peaked at 98%. Heffernan, Shrivastava et al., (2017) reported that provided a certain thickness threshold is not exceeded, thick biofilms will have

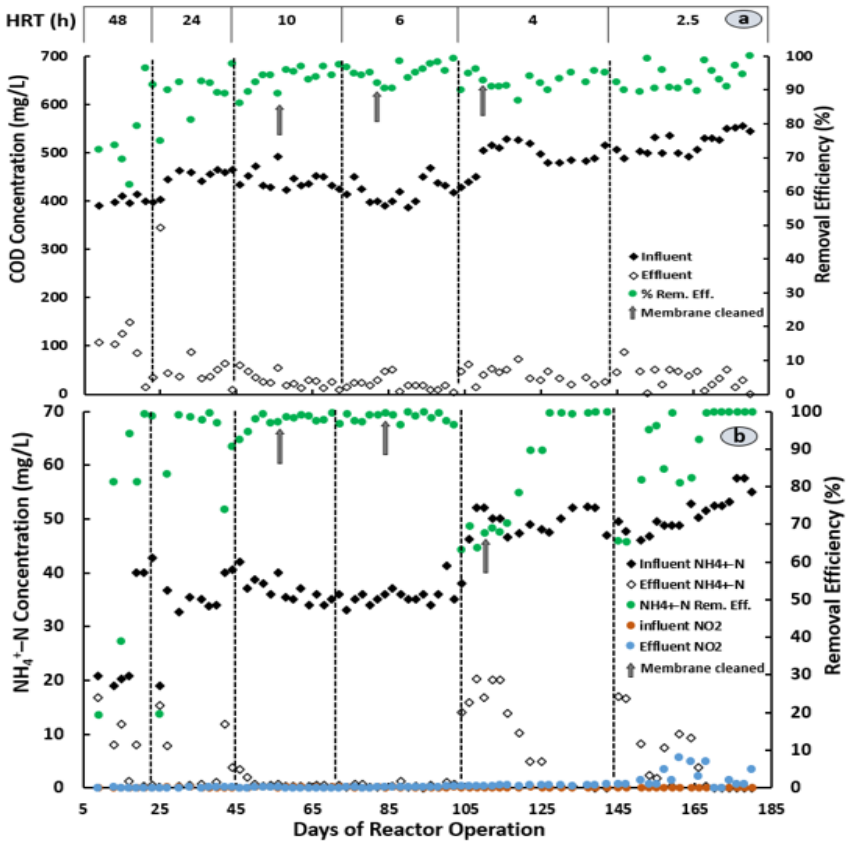


Figure 4.1. a and b. MABR performance: (a) COD removal efficiency and (b) NH_4^+-N removal efficiency of the reactor at different HRTs of operation. Arrows represent the time of membrane biofilm cleaning.

higher nitrogen removal rates which could be due to the large volume-to-surface area ratio and a robust, resilient, and stable microbial community obtainable in the MABR system (Suarez, Piculell et al. 2019). However, as the system load was doubled to 0.60 gN/m^2/d (HRT: 4 h), NH_4^+-N removal efficiency reduced by over 30%, and the DO in the reactor decreased below 0.2 mg/L, necessitating another membrane cleaning. Reactor performance picked up shortly after membrane cleaning, and increased consistently, peaking at 99% removal efficiency. The MABR resiliency was further stretched by increasing the SLR to 1.0 gN/m^2/d (HRT: 2.5 h), which resulted in a sharp drop (35%) in the MABR performance. However, reactor performance began to accelerate consistently four days later without another membrane cleaning. Reactor performance eventually reached nearly 100%, possibly due to the non-aggressive membrane cleaning applied. Non-aggressive membrane

cleaning removes the outer biofilm, leaving the inner biofilm layers to control heterotrophic bacteria (HB) proliferation at the membrane surface, and does not interfere with nitrifying microbes at the base of the biofilm.

4.3.1.3. Total inorganic nitrogen removal

A high DO concentration (> 2 mg/L) prevented the formation of anoxic/anaerobic regimes within the biofilms in the early stages of MABR operation, severely limiting denitrification. However, at an HRT of 10 h, the TIN removal efficiency was almost 30% (Figure 4.2). The effluent NH_4^+-N concentration was consistently close to 0 mg/L, which can be attributed mainly to the presence of well-developed nitrifiers that exhibited a high capacity for ammonia oxidation at the biofilm base. However, effluent NO_2^--N was negligible at this condition, while the average effluent NO_3^--N was 13 mg/l, indicating poor denitrification. At an HRT of 4 h, with a bulk liquid DO of 0.3 - 0.4 mg/L, the TIN removal efficiency increased to 85%. The effluent NH_4^+-N concentration remained negligible, whereas the average effluent NO_3^--N declined by 80% to 2.6 mg/L, showing improved denitrification. Further HRT reduction to 2.5 h, led to a 1% drop in the TIN removal efficiency, while the effluent NO_3^--N increased from 2.6 mg/L to 4 mg/L and the effluent NO_2^--N increased from 0.9 mg/L to 2.5 mg/L due to a drop in the DO. This performance level was sustained until the end of the study. The occurrence of several processes simultaneously indicates biofilm stratification. The biofilm thickness control strategy applied in this study effectively sustained the stratified biofilm developed and led to a high TIN removal efficiency from municipal wastewater.

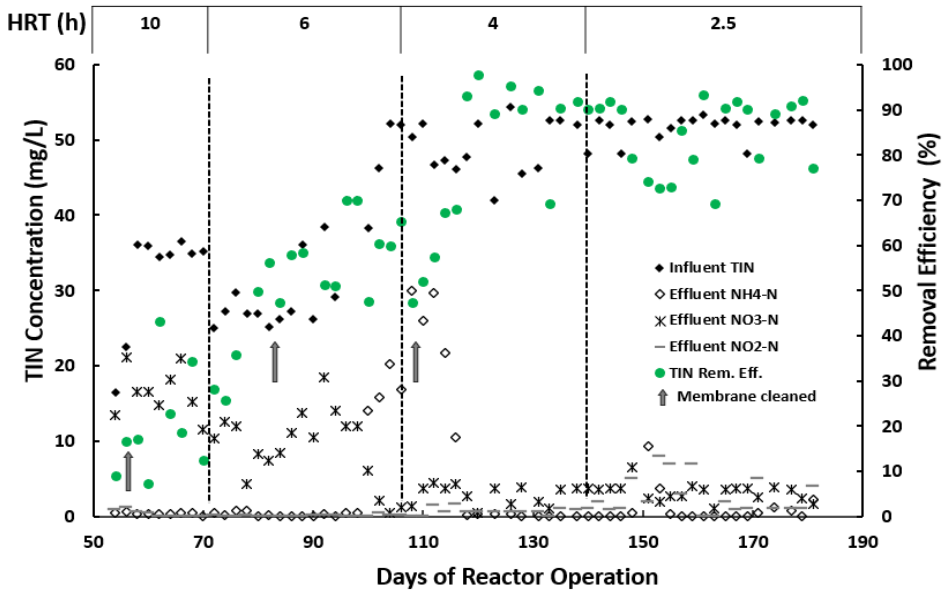


Figure 4.2. Total inorganic nitrogen removal in the MABR with respect to the HRT. Arrows represent the time of membrane biofilm cleaning.

4.3.2. DO concentration profile

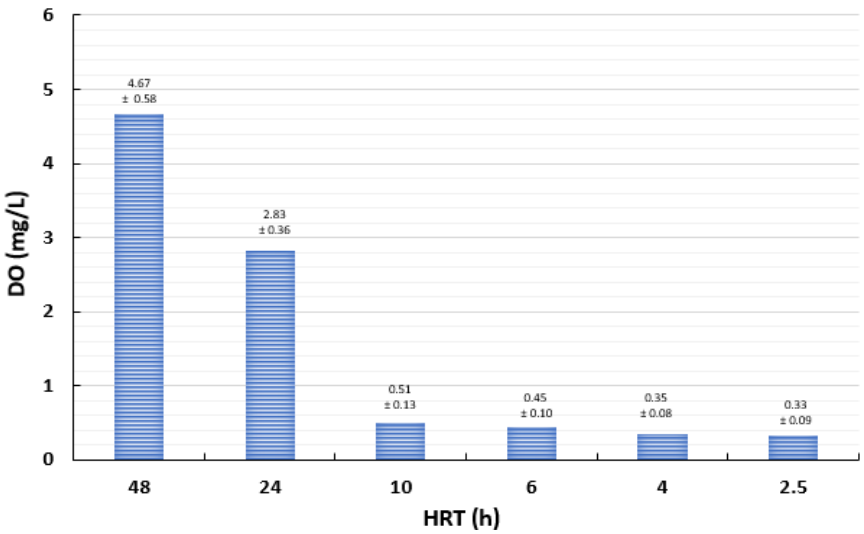


Figure 4.3. Average dissolved oxygen concentration as a function of time at different HRTs of MABR operation.

Figure 4.3 shows the DO concentration gradient in the MABR over the duration of the study. Effluent and bulk DO concentrations were considered identical due to the system homogeneity provided by biomass recirculation. At the start-up stage with an HRT of 48h, the effluent DO

concentration was ~ 4.7 mg/L; the effluent DO concentration dropped to ~ 2.8 mg/L over 20 days, indicating microbial activities and biofilm formation on the membrane surfaces. Fitzgerald et al., (2015) reported that optimal DO concentration for nitrifiers is in the range of 0.3- 4.0 mg/L, while Sun et al., (2022) suggested appreciable amount of biofilm develops on membrane surfaces occurs between 2- 4 weeks. At HRT of 10 h, due to the several oxygen utilization processes occurring within the biofilm, the bulk DO decrease to anoxic levels (~ 0.5 mg/L). Thereafter, the decrease of DO concentration became gradual. The reactor maintained high performance, with average organic carbon and ammonia removal efficiencies > 90 and 99%, respectively, at an HRT of 2.5 h. With the increase in biofilm thickness, a decreasing DO concentration in the inner layers of the biofilm created both anoxic and anaerobic conditions within the system; evidence for this was the high relative abundance of both anoxic and anaerobic microbes.

4.3.3. Biofilm thickness and density

The common means of controlling MABR biofilm thickness is through air-bubble induced sloughing (Semmens 2005, Syron, Semmens and Casey 2015, Peeters, Long et al. 2017, Bunse, Orschler et al. 2020). In this study, the membranes were cleaned with water at stages 3, 4, and 5, or whenever the dissolved oxygen (DO) dropped below 0.2 mg/L. Biofilm thickness and performance stabilized after day 110. Studies have suggested that active biofilm cleaning is required to prevent biofilm overgrowth (Stricker, Lossing et al. 2011). The average biofilm thickness in this study was determined at the end of stage 6 to be 0.49 mm (Table 4.2), which is approximately the minimum biofilm thickness that must be attained for nitritation to occur in a one-stage MABR (Wagner, Daigger and Love 2022). This value is consistent with other MABR studies that applied loading rates of 5-15 $gCOD/m^2d$ and 0.3-6 $gNH_3\text{-}N/m^2d$, and operated with a biofilm thickness control mechanism. Reported biofilm thicknesses for these studies ranged from 0.2-0.5 mm (Stricker,

Lossing et al. 2011, Bunse, Orschler et al. 2020, Taşkan, Hasar and Lee 2020). Studies without thickness control mechanisms have reported biofilm thicknesses of 0.8-3.5 mm (Shaowei, Fenglin et al. 2008, Wu, Wu et al. 2019, Sanchez-Huerta, Fortunato et al. 2022). Without thickness control, biofilms are susceptible to overgrowth that can result in irregular surfaces. Thick biofilms are easily detachable and can compromise system performance (Semmens, Dahm et al. 2003, Pellicer-Nàcher, Franck et al. 2014, He, Wagner et al. 2021).

But biofilm thickness control has pitfalls. For instance, scouring can lead to performance perturbations (Syron, Semmens and Casey 2015, He, Wagner et al. 2021). Bunse, Orschler et al, (2020) reported short-term losses of 50-100% of the total nitrogen (TN) removal capacity. To control biofilm thickness, we intermittently washed the membrane bundle with clean water. We did not observe extended performance upsets. The average biofilm density of 17.6 g/L obtained in this study compared favourably with the 19 g/L biofilm density reported by Wu, Wu et al., (2019). However, different studies with similar operating conditions did not always produce consistent results. Biofilm densities of 3-64 g/L have been reported. Shaowei, Fenglin et al. (2008) reported 3.7 g/L, Bunse, Orschler et al. (2020) reported 40g/L, and Pellicer-Nàcher and Smets (2014) reported 64 g/L. Thus, multiple factors besides substrate loading rates contribute to changes in biofilm density. Other factors that have been shown to contribute to biofilm density are fluid flow velocity (Casey, Glennon and Hamer 2000), shear rate and DO concentration (Pellicer-Nàcher and Smets 2014), and COD concentration (Semmens 2005).

Table 4.2. Biofilm Density Estimated at The End of This Study

Component	Value
Average biofilm mass (g)	2.392 ± 0.044
Weight differential (g)	0.012 ± 0.001
Membrane length (m)	0.220
Membrane outer diameter (m)	0.0022
Membrane surface area (m^2)	0.042
Membrane count	30
Average biofilm thickness (mm)	0.49
Areal density (g/m^2)	8.6 ± 0.7
Density (g/L)	17.4 g/L ± 0.73

4.3.4. Analyses of viable cells and dead cells

Microbial colonies with predominantly viable cells were revealed in the MABR biofilms by fluorescence microscopy (Figure 4.4). The deep green colour in Figure 4.4 suggests that the cleaning mechanism was non-destructive to a large portion of the biofilm, which aligns with the high performance reported in this study. Organic contaminant breakdown can be achieved only by active microbes with viable cells.

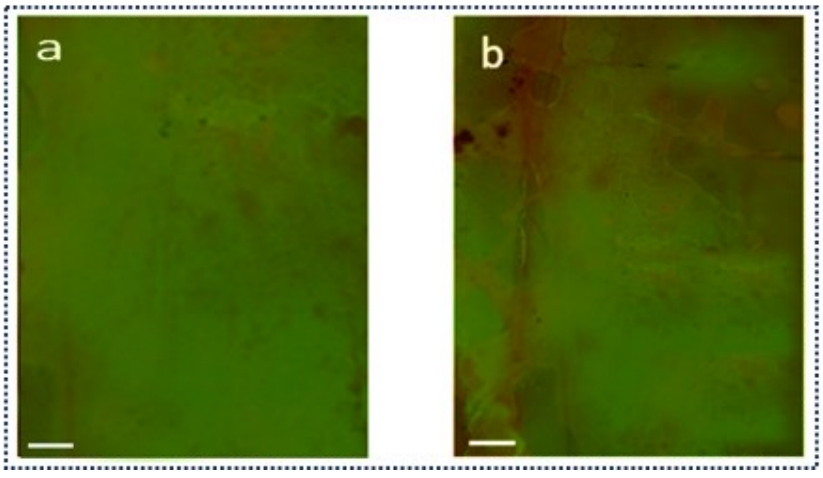


Figure 4.4. Fluorescence microscopy image showing the distribution of live (green) and dead (red) cells in the membrane biofilm, taken from different locations on day 185 at an HRT of 2.5 h. Image is at 200x magnification and 1mm scale.

4.3.5. EPS composition

Components of the extracellular polymeric substances (EPS) in the biofilm were extracted on day 185 at an HRT of 2.5 h. The biomass concentration was determined to be 0.13 gVSS/L; polysaccharide and protein concentrations were 6.75 and 12.96 mg/L, respectively. The ~ 50% higher protein fraction agrees with studies that reported more abundant protein than carbohydrate in EPS (Hoa, Nair and Visvanathan 2003, Liang, Li et al. 2010, Awolusi, Kumari and Bux 2015). This is probably due to the rapid hydrolysis of polysaccharides (Zhou, Li et al. 2022). The biofilm in this study produced higher quantities of protein and carbohydrate than the biofilms reported in Li et al., (2010), and in Zhou et al., (2022), both of which were cultivated from synthetic wastewater with bicarbonate as a sole carbon source. Studies have suggested that the composition of EPS and the properties of EPS have a greater influence than the quantity of EPS on microbial community aggregation and function, as each EPS component plays a specific role (Shi, Huang et al. 2017). Moreover, EPS vary in quantity and composition as a result of environmental factors and operational conditions (Sun, Li et al. 2022). EPS accumulation promotes microbial aggregation, and therefore improves reactor performance (Shi, Huang et al. 2017, Sun, Li et al. 2022, Zhou, Li et al. 2022). This aligns with the maintenance of a high COD removal and the efficient removal of ammonia and inorganic nitrogen achieved in this study.

4.3.6. Microbial community analysis

The microbes involved in pollutant degradation are critical to the success of MABR technology. A high-throughput sequencing of 16S rRNA gene amplicons found that the majority of microbes in the MABR were from the ubiquitous Proteobacteria phylum. Proteobacteria play a vital role in organic compound oxidation and nutrients removal from wastewater (Iorhemen, Ukaigwe et al. 2022). Subdominant phyla included: Bacteroidetes, Actinobacteria, Planctomycetes, and Firmicutes. From the Bacteroidetes phylum, the genera *Cytophagia* and *Flavobacteriia* had a high

relative abundance. Within the Alphaproteobacteria class, Sphingomonadales, Caulobacteraceae, Xanthobacteraceae, and Rhodospirillaceae, also had a high relative abundance, whereas the abundances of Comamonadacea and Rhodocyclaceae from the Betaproteobacteria class were relatively low. However, for the Gammaproteobacteria class, the relative abundance of prominent families like Xanthomodonadeceace and Pseudomonadaceae varied by different HRTs.

Several known biofilm formers including Comamonadeae, Xanthononadaceae, Pseudomonadeceae, and Enterobacteriaceae (Kelly, London et al. 2021) were also prominent at the family level. At the genus level, there was a significant number of non-putative municipal wastewater microbes, indicating that there might be other less known microbial genera involved in ammonia oxidation and nitrogen removal. Some microbes that were undetectable in the inoculum were found to increase in relative abundance over the course of the experiment, whereas others that were detectable in the inoculum decreased over time. The biofilm thickness control strategy employed in this study efficiently controlled the relative abundance of all of the microbes in the microbial community, enabling the reactor to maintain a stable performance throughout the study duration.

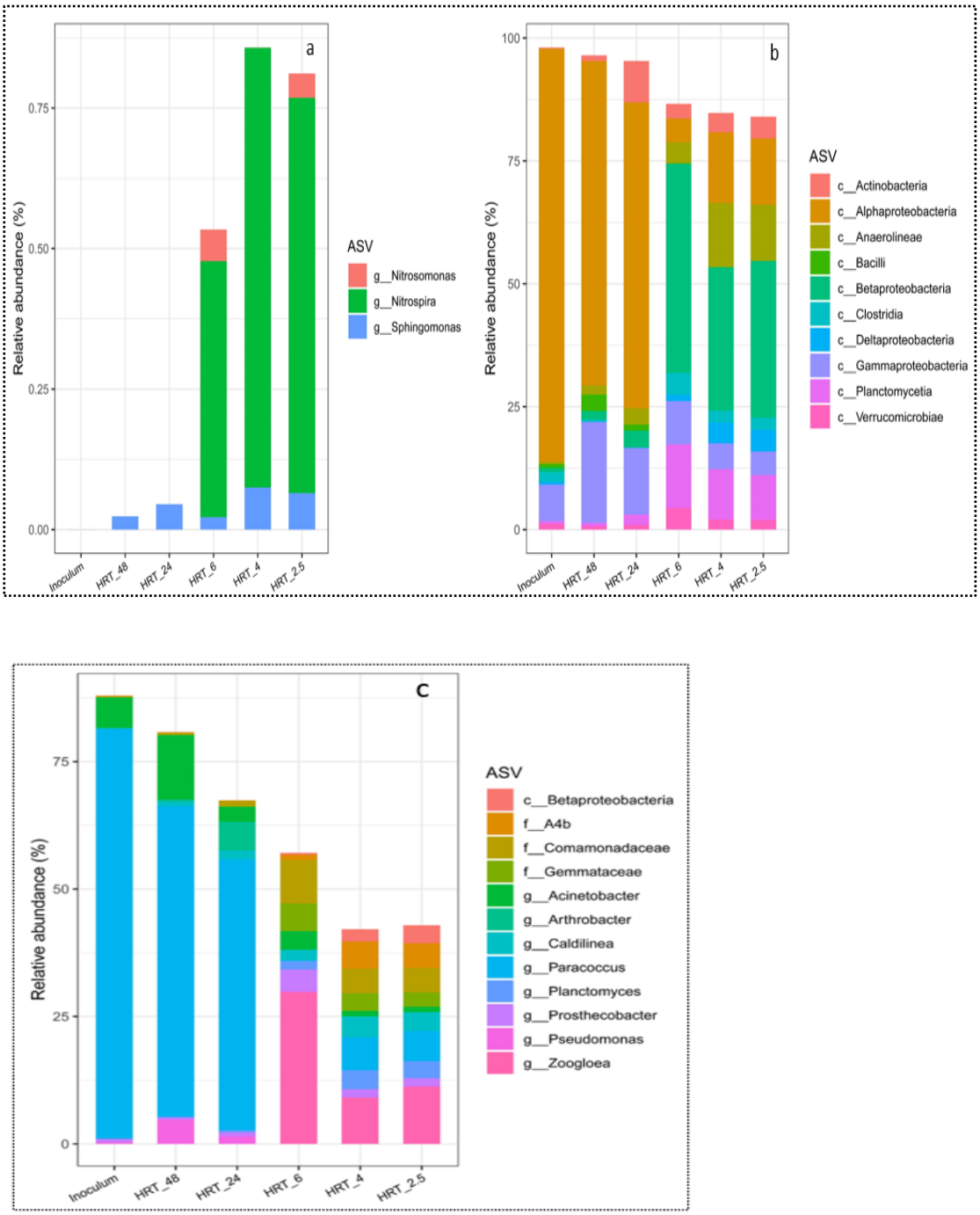


Figure 4.5a, b and c. (a) High-throughput sequencing of 16S rRNA gene amplicons showing the distribution of the microbial community in the biofilm at HRTs of 48, 24, 6, 4, and 2.5 h. Ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) at genus level. (b) The most abundant amplicon sequence variants (ASVs) at HRTs of 48, 24, 6, 4, and 2.5 h at class level. (c) genus level classification.

4.3.6.1. Nitrifying bacterial population dynamics

The richness, diversity, and structure of the microbial communities within a reactor can be affected by operational conditions, inoculum properties, and the biofilm thickness control strategy (Terada, Lackner et al. 2010, Bassin, Kleerebezem et al. 2012, Liu and Wang 2013, Tian, Zhao et al. 2015, Bunse, Orschler et al. 2020, Li, Bao et al. 2023). Figure 4.5a shows the relative abundance of detectable nitrifying bacteria in the MABR biofilm at the genus level. These include AOBs (*Nitrosomonas and Sphingomonas*) and NOBs (*Nitrospira*) (Mota, Ridenoure et al. 2005, Krishna, Sathasivan and Ginige 2013, Fitzgerald, Camejo et al. 2015, Zhou, Li et al. 2020).

Whereas *Sphingomonas* was undetected in the inoculum, its relative abundance increased between HRTs of 48 and 24 h, this might have resulted from conducive aerobic conditions within the system. Liu and Wang (2013) and Li, Bao et al., (2023) reported that DO concentrations ranging from 0.3 to 4.0 are optimal for nitrifiers. At an HRT of 6 h, the relative abundance of *Sphingomonas* declined. The decline of *Sphingomonas* might have been due to an increased biomass in the reactor that led to a drop in the DO to anoxic levels (Figure 4.3). However, when the HRT was further reduced to 4 h, and finally to 2.5 h, the relative abundance of *Sphingomonas* increased and stabilized over time. A variation in the relative abundance of microorganisms as operational conditions change might indicate a high sensitivity of the microorganisms to the changing operating conditions. However, nitrifiers have been reported to consistently display high adaptive capacities in response to environmental and operational conditions (Awolusi, Kumari and Bux 2015). An ability to proliferate in non-optimal conditions would explain the presence of nitrifiers in both inoculum and biofilm microbial assemblages at almost all HRTs. *Sphingomonas* have been reported to degrade complex organic compounds and to produce polysaccharide that enhances biofilm formation (Czieborowski, Hübenthal et al. 2020, Kelly, London et al. 2021).

Nitrosomonas populations became detectable at HRT of 6 h; they declined significantly at HRT of 4 h, possibly due to increasingly anoxic conditions (Figure 4.3), and eventually stabilized at HRT of 2.5 h. As R-strategist species (Gilbert, Agrawal et al. 2014), the higher DO concentrations at earlier stages of the study favoured their proliferation; as the dissolved oxygen decreased over the operational time, the conditions became less favourable. However, the increase in *Nitrosomonas* at HRT of 2.5 h can be ascribed to the capacity of MABR biofilms to provide a protective niche for aerobic microbes (Nerenberg 2016).

Nitrospira, a slow-growing K-strategist species also became detectable at a later stage (HRT of 6 h). Although K-strategist species have a slower growth rate than R-strategist species (Gilbert, Agrawal et al. 2014, Bunse, Orschler et al. 2020), the former consistently increased in relative abundance over time, as the stable biofilm provided protection against washout.

Two types of strategists are typically present in wastewater treatment ecosystems, R-strategists (e.g., *Nitrosomonas*) with fast growth rates and low substrate affinities and K-strategists (e.g., *Nitrosospira*) with slower growth rates. R-strategists can achieve high pollutant loading rates, while, K-strategists can decrease the pollutant concentration to low level (Wu and Yin 2020, Yin, Sun et al. 2022). Systems with high substrate gradients like the MABR are able to provide specialized niches for both R and K-strategists and thus simultaneously achieve high pollutant removal rate and removal efficiency (Wu and Yin 2020).

This MABR demonstrated high pollutants removal efficiency due to the ability of the system to maintain high relative abundance of multifunctional microbes at each stage of the study (Figure 4.5a, b and c).

4.3.6.2. Bacterial population dynamics at class and genus levels

At class level, the dominant communities included Alphaproteobacteria (4.8-62%), Betaproteobacteria (3.4-42%), and Gammaproteobacteria (4.5-13%), all of which belong to Proteobacteria. Members of the Proteobacteria phylogenetic group have extreme metabolic diversity and are regarded as universally important for denitrification and organic compound biodegradation (Zhou, Li et al. 2020, Iorhemen, Ukaigwe et al. 2022). The relative abundance of Alphaproteobacteria in the reactor consistently decreased as the HRT was reduced from 48 h to 6 h, in contrast to Betaproteobacteria which increased. However, Alphaproteobacteria and Betaproteobacteria are broad classifications and many factors can modulate their relative abundance. Sub-dominant classes include; Planctomycetia (2-19%), Saprospirae (0.15-1.62%), and Actinobacteria (3.0-8.4%), all of which have nitrogen removal capacities (Tian, Zhao et al. 2015, Lin, Zhang et al. 2016, Zhang, Yu et al. 2018).

The relative abundance of Planctomycetia and Actinobacteria increased over time as the HRT decreased from 48 h — 2.5 h, whereas Saprospirae did not maintain a consistent pattern. Which imply that multiple factors including; SLR, substrates and DO concentrations, biofilm thickness, affected the relative abundance of the microbial communities within the reactor. Figure 4.5c presents the genus levels of other nitrifying bacteria in the inoculum and the biofilms at HRTs of 48, 24, 6, 4, and 2.5 h. The dominant bacteria groups included: *Pseudomonas* (0.6-1.47%), *Acinetobacter* (0.62-3.6%), *Zoogloea* (0 - 29.8%), *Comamonas* (0.15 - 0.3%), and *Rhodococcus* (0.7-15.2%); all are known to be aerobic denitrifying bacteria (ADB) (Krishna, Sathasivan and Ginige 2013, Lin, Zhang et al. 2016, Chen, Cao et al. 2022).

Dominant anaerobic denitrifying bacteria (AnDB) included *Paracoccus* (0.2-53.1%) and *Thauera (0-0.9%). Thauera* are associated with nitrification and denitrification under both aerobic and anaerobic conditions (Shinoda, Sakai et al. 2004, Sun, Li et al. 2022).

Planctomyces are often present during anaerobic ammonium oxidation (anammox) under conditions of limited oxygen (Gong, Yang et al. 2007). Planctomyces (0.38-1.68%) were found in low relative abundance in the MABR, but increased over time. Relative to heterotrophs, anammox bacteria have slower growth rates (Wang, Liang et al. 2021). The increase in the relative abundance of anammox bacteria may have been triggered by the increase in the anoxic condition within the MABR. Operational conditions can affect both the biodiversity and the stability of microbial populations (Mielczarek, Nguyen et al. 2013, Zhang, Yu et al. 2018). Tian et al. (2015) reported that the bacterial community shifted as the influent quality changed in an MABR treating synthetic domestic wastewater; however, the relative abundances of the dominant microbial phylogenetic groups within the reactor did not change. Egli et al., (2003) reported mostly identical microbial communities at specific operating conditions in five identical nitritation reactors. Cydzik-Kwiatkowska and Zielińska, (2016) monitored the bacterial community in activated sludge from a full-scale municipal wastewater treatment plant for one year and reported that although the total bacterial community in the activated sludge changed moderately with the passage of time, the ammonia-oxidising bacteria community did not change over the study duration (Cydzik-Kwiatkowska and Zielińska 2016).

In the present study, although the biodiversity of the microbial community changed noticeably, the relative abundance of the microbial community was fairly constant (HRTs of 4 h and 2.5 h) due to a minimal loss of biofilm mass.

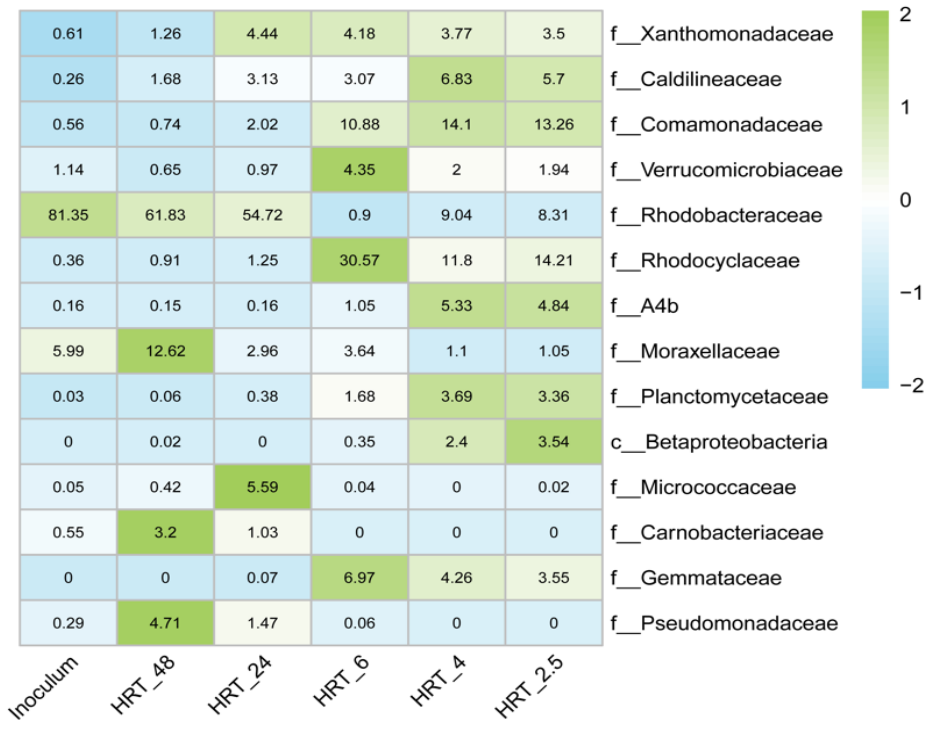


Figure 4.6. The 13 most abundant ASVs in the biofilm at HRTs of 48, 24, 6, 4, and 2.5 h at family level.

4.4. Conclusion

In the present study, multifunctional biofilms with adequate thickness to support optimum performance for treatment of medium-strength MWW over extended operation periods were developed in an MABR. Using a simple membrane cleaning procedure, the equilibrium between biofilm growth and biofilm loss were balanced by gently detaching loosely bound biofilm layers from the membrane, while the more cohesive layers with sufficient microbes to maintain microbial activities and prevent process interruption were retained. Under the operating conditions established, mean removal efficiencies of 92 %, 99 % and 84 % respectively were obtained for COD, NH_4^+-N and TIN at HRT of 2.5h. Septicity that often leads to prolonged reactor downtimes in MABR systems was controlled by initiating membrane cleaning as bulk DO drop to set-point of 0.2mg/L. As biofilm must work optimally for extended periods for high performance to be maintained, application of this cleaning mechanism in MABR operations will ensure the

maintenance of biofilm with adequate thickness to sustain uninterrupted operation. This work will expand the current knowledge on MABR biofilm thickness management and increase the potential for wider adoption of the technology.

4.5. References

Anh-Vu, N., Yun-Je, L., Masumi, K. & Visvanathan, C. 2022. Effects of membrane relaxation rate on performance of pilot-scale membrane aerated biofilm reactors treating domestic wastewater. *Environmental Research,* 211**,** 113003.

APHA AWWA, W. 1998. Standard methods for the examination of water and wastewater ^{2}0th edition. *American Public Health Association, American Water Work Association, Water Environment Federation, Washington, DC*.

Awolusi, O., Kumari, S. & Bux, F. 2015. Ecophysiology of nitrifying communities in membrane bioreactors. *International Journal of Environmental Science and Technology,* 12**,** 747-762.

Bassin, J., Kleerebezem, R., Rosado, A., Van Loosdrecht, M. M. & Dezotti, M. 2012. Effect of different operational conditions on biofilm development, nitrification, and nitrifying microbial population in moving-bed biofilm reactors. *Environmental Science & Technology,* 46**,** 1546-1555.

Bunse, P., Orschler, L., Agrawal, S. & Lackner, S. 2020. Membrane aerated biofilm reactors for mainstream partial nitritation/anammox: Experiences using real municipal wastewater. *Water Research X,* 9**,** 100066.

Callahan, B. J., Mcmurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A. & Holmes, S. P. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature methods,* 13**,** 581-583.

Casey, E., Glennon, B. & Hamer, G. 2000. Biofilm development in a membrane‐aerated biofilm reactor: Effect of flow velocity on performance. *Biotechnology and Bioengineering,* 67**,** 476-486.

Chen, R., Cao, S., Zhang, L. & Zhou, Y. 2022. NOB suppression strategies in a mainstream membrane aerated biofilm reactor under exceptionally low lumen pressure. *Chemosphere,* 290**,** 133386.

Côté, P., Peeters, J., Adams, N., Hong, Y., Long, Z. & Ireland, J. 2015. A new membrane-aerated biofilm reactor for low energy wastewater treatment: Pilot results. *Proceedings of the Water Environment Federation,* 13**,** 4226-4239.

Cydzik-Kwiatkowska, A. & Zielińska, M. 2016. Bacterial communities in full-scale wastewater treatment systems. *World Journal of Microbiology and Biotechnology,* 32**,** 1-8.

Czieborowski, M., Hübenthal, A., Poehlein, A., Vogt, I. & Philipp, B. 2020. Genetic and physiological analysis of biofilm formation on different plastic surfaces by Sphingomonas sp. strain S2M10 reveals an essential function of sphingan biosynthesis. *Microbiology,* 166**,** 918-935.

Da Silva, T. S., Matsumoto, T., Dos Anjos, M. L. & Albertin, L. L. 2018. Organic matter removal in a membrane-aerated biofilm reactor. *Journal of Environmental Engineering,* 144**,** 04018057.

Dodds, W. K. & Welch, E. B. 2000. Establishing nutrient criteria in streams. *Journal of the North American Benthological Society,* 19**,** 186-196.

Fitzgerald, C. M., Camejo, P., Oshlag, J. Z. & Noguera, D. R. 2015. Ammonia-oxidizing microbial communities in reactors with efficient nitrification at low-dissolved oxygen. *Water research,* 70**,** 38-51.

Frølund, B., Palmgren, R., Keiding, K. & Nielsen, P. H. 1996. Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water research,* 30**,** 1749-1758.

Gilbert, E. M., Agrawal, S., Brunner, F., Schwartz, T., Horn, H. & Lackner, S. 2014. Response of different Nitrospira species to anoxic periods depends on operational DO. *Environmental science & technology,* 48**,** 2934-2941.

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**,** 776-784.

Hall-Stoodley, L., Costerton, J. W. & Stoodley, P. 2004. Bacterial biofilms: from the natural environment to infectious diseases. *Nature reviews microbiology,* 2**,** 95-108.

He, H., Wagner, B. M., Carlson, A. L., Yang, C. & Daigger, G. T. 2021. Recent progress using membrane aerated biofilm reactors for wastewater treatment. *Water Science and Technology,* 84**,** 2131-2157.

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**,** 285-297.

Hoa, P., Nair, L. & Visvanathan, C. 2003. The effect of nutrients on extracellular polymeric substance production and its influence on sludge properties. *Water Sa,* 29**,** 437-442.

Houweling, D. & Daigger, G. T. 2019. *Intensifying Activated Sludge Using Media-Supported Biofilms*, CRC Press.

Hu, X. B., Xu, K., Wang, Z., Ding, L. L. & Ren, H. Q. 2013. Characteristics of biofilm attaching to carriers in moving bed biofilm reactor used to treat vitamin C wastewater. *Scanning,* 35**,** 283-291.

Hwang, J. H., Cicek, N. & Oleszkiewicz, J. A. 2009. Membrane biofilm reactors for nitrogen removal: state-of-the-art and research needs. *Water Science and Technology,* 60**,** 2739-2747.

Iorhemen, O. T., Ukaigwe, S., Dang, H. & Liu, Y. 2022. Phosphorus Removal from Aerobic Granular Sludge: Proliferation of Polyphosphate-Accumulating Organisms (PAOs) under Different Feeding Strategies. *Processes,* 10**,** 1399.

Janczewski, L. & Trusek-Holownia, A. 2016. Biofilm-based membrane reactors–selected aspects of the application and microbial layer control. *Desalination and Water Treatment,* 57**,** 22909-22916.

Kelly, J. J., London, M. G., Mccormick, A. R., Rojas, M., Scott, J. W. & Hoellein, T. J. 2021. Wastewater treatment alters microbial colonization of microplastics. *PloS one,* 16**,** e0244443.

Khalid, S., Shahid, M., Bibi, I., Sarwar, T., Shah, A. H. & Niazi, N. K. 2018. A review of environmental contamination and health risk assessment of wastewater use for crop irrigation with a focus on low and high-income countries. *International journal of environmental research and public health,* 15**,** 895.

Krishna, K. B., Sathasivan, A. & Ginige, M. P. 2013. Microbial community changes with decaying chloramine residuals in a lab-scale system. *Water research,* 47**,** 4666-4679.

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 and Biotechnology,* 33**,** 315-323.

Li, X., Bao, D., Zhang, Y., Xu, W., Zhang, C., Yang, H., Ru, Q., Wang, Y.-F., Ma, H. & Zhu, E. 2023. Development and application of membrane aerated biofilm reactor (MABR)—A review. *Water,* 15**,** 436.

Liang, Z., Li, W., Yang, S. & Du, P. 2010. Extraction and structural characteristics of extracellular polymeric substances (EPS), pellets in autotrophic nitrifying biofilm and activated sludge. *Chemosphere,* 81**,** 626-632.

Lin, J., Zhang, P., Li, G., Yin, J., Li, J. & Zhao, X. 2016. Effect of COD/N ratio on nitrogen removal in a membrane-aerated biofilm reactor. *International Biodeterioration & Biodegradation,* 113**,** 74-79.

Liu, G. & Wang, J. 2013. Long-term low DO enriches and shifts nitrifier community in activated sludge. *Environmental science & technology,* 47**,** 5109-5117.

Luo, Y.-H., Chen, R., Wen, L.-L., Meng, F., Zhang, Y., Lai, C.-Y., Rittmann, B. E., Zhao, H.-P. & Zheng, P. 2015. Complete perchlorate reduction using methane as the sole electron donor and carbon source. *Environmental science & technology,* 49**,** 2341-2349.

Ma, Y., Piscedda, A., Veras, A. D. L. C., Domingo-Félez, C. & Smets, B. F. 2022. Intermittent aeration to regulate microbial activities in membrane-aerated biofilm reactors: Energy-efficient nitrogen removal and low nitrous oxide emission. *Chemical Engineering Journal,* 433**,** 133630.

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

Mccarty, P. L., Bae, J. & Kim, J. 2011. Domestic wastewater treatment as a net energy producer–can this be achieved? : ACS Publications.

Mcdonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., Desantis, T. Z., Probst, A., Andersen, G. L., Knight, R. & Hugenholtz, P. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME journal,* 6**,** 610-618.

Mielczarek, A. T., Nguyen, H. T. T., Nielsen, J. L. & Nielsen, P. H. 2013. Population dynamics of bacteria involved in enhanced biological phosphorus removal in Danish wastewater treatment plants. *Water research,* 47**,** 1529-1544.

Mota, C., Ridenoure, J., Cheng, J. & De Los Reyes Iii, F. L. 2005. High levels of nitrifying bacteria in intermittently aerated reactors treating high ammonia wastewater. *FEMS Microbiology Ecology,* 54**,** 391-400.

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

Peeters, J., Long, Z., Houweling, D., Côté, P., Daigger, G. T. & Snowling, S. Nutrient removal intensification with MABR–developing a process model supported by piloting. Nutrient Removal and Recovery Symposium 2017, 2017. Water Environment Federation.

Pellicer-Nàcher, C. & Smets, B. F. 2014. Structure, composition, and strength of nitrifying membrane-aerated biofilms. *water research,* 57**,** 151-161.

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**,** 32-43.

Pérez-Calleja, P., Clements, E. & Nerenberg, R. 2022. Enhancing ammonium oxidation fluxes and nitritation efficiencies in MABRs: a modeling study. *Environmental Science: Water Research & Technology,* 8**,** 358-374.

Picioreanu, C., Van Loosdrecht, M. C. & Heijnen, J. J. 2001. Two‐dimensional model of biofilm detachment caused by internal stress from liquid flow. *Biotechnology and bioengineering,* 72**,** 205-218.

Pierce, J. & Suelter, C. 1977. An evaluation of the Coomassie brilliant blue G-250 dye-binding method for quantitative protein determination. *Analytical Biochemistry,* 81**,** 478-480.

Rout, P. R., Shahid, M. K., Dash, R. R., Bhunia, P., Liu, D., Varjani, S., Zhang, T. C. & Surampalli, R. Y. 2021. Nutrient removal from domestic wastewater: A comprehensive review on conventional and advanced technologies. *Journal of Environmental Management,* 296**,** 113246.

Sanchez-Huerta, C., Fortunato, L., Leiknes, T. & Hong, P.-Y. 2022. Influence of biofilm thickness on the removal of thirteen different organic micropollutants via a Membrane Aerated Biofilm Reactor (MABR). *Journal of Hazardous Materials,* 432**,** 128698.

Semmens, M. 2005. Membrane technology: pilot studies of membrane-aerated bioreactors.

Semmens, M. J., Dahm, K., Shanahan, J. & Christianson, A. 2003. COD and nitrogen removal by biofilms growing on gas permeable membranes. *Water Research,* 37**,** 4343-4350.

Shaowei, H., Fenglin, Y., Cui, S., Zhang, J. & Tonghua, W. 2008. Simultaneous removal of COD and nitrogen using a novel carbon-membrane aerated biofilm reactor. *Journal of Environmental Sciences,* 20**,** 142-148.

Shi, Y., Huang, J., Zeng, G., Gu, Y., Chen, Y., Hu, Y., Tang, B., Zhou, J., Yang, Y. & Shi, L. 2017. Exploiting extracellular polymeric substances (EPS) controlling strategies for performance enhancement of biological wastewater treatments: an overview. *Chemosphere,* 180**,** 396-411.

Shinoda, Y., Sakai, Y., Uenishi, H., Uchihashi, Y., Hiraishi, A., Yukawa, H., Yurimoto, H. & Kato, N. 2004. Aerobic and anaerobic toluene degradation by a newly isolated denitrifying bacterium, Thauera sp. strain DNT-1. *Applied and Environmental Microbiology,* 70**,** 1385-1392.

Siriweera, W. B. S., Yun-Je, L., Masumi, K. & Visvanathan, C. 2021. Organic matter and total nitrogen removal from wastewater using a pilot-scale membrane-aerated biofilm reactor. *Bioresource Technology Reports,* 15**,** 100817.

Stricker, A. E., Lossing, H., Gibson, J. H., Hong, Y. & Urbanic, J. C. 2011. Pilot Scale Testing of A New Configuration of The Membrane Aerated Biofilm Reactor (MABR) to Treat High‐Strength Industrial Sewage. *Water Environment Research,* 83**,** 3-14.

Suarez, C., Piculell, M., Modin, O., Langenheder, S., Persson, F. & Hermansson, M. 2019. Thickness determines microbial community structure and function in nitrifying biofilms via deterministic assembly. *Scientific reports,* 9**,** 1-10.

Sun, Z., Li, Y., Li, M., Wang, N., Liu, J., Guo, H. & Li, B. 2022. Steel pickling rinse wastewater treatment by two-stage MABR system: Reactor performance, extracellular polymeric substances (EPS) and microbial community. *Chemosphere,* 299**,** 134402.

Syron, E. & Casey, E. 2008. Membrane-aerated biofilms for high rate biotreatment: performance appraisal, engineering principles, scale-up, and development requirements. *Environmental science & technology,* 42**,** 1833-1844.

Syron, E., Semmens, M. J. & Casey, E. 2015. Performance analysis of a pilot-scale membrane aerated biofilm reactor for the treatment of landfill leachate. *Chemical Engineering Journal,* 273**,** 120-129.

Taşkan, B., Hasar, H. & Lee, C. H. 2020. Effective biofilm control in a membrane biofilm reactor using a quenching bacterium (Rhodococcus sp. BH4). *Biotechnology and Bioengineering,* 117**,** 1012-1023.

Tay, J.-H., Liu, Q.-S. & Liu, Y. 2002. Aerobic granulation in sequential sludge blanket reactor. *Water Science and Technology,* 46**,** 13-18.

Terada, A., Hibiya, K., Nagai, J., Tsuneda, S. & Hirata, A. 2003. Nitrogen removal characteristics and biofilm analysis of a membrane-aerated biofilm reactor applicable to high-strength nitrogenous wastewater treatment. *Journal of bioscience and bioengineering,* 95**,** 170-178.

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

Tian, H.-L., Zhao, J.-Y., Zhang, H.-Y., Chi, C.-Q., Li, B.-A. & Wu, X.-L. 2015. Bacterial community shift along with the changes in operational conditions in a membrane-aerated biofilm reactor. *Applied microbiology and biotechnology,* 99**,** 3279-3290.

Ukaigwe, S., Zhou, Y., Shaheen, M. & Liu, Y. 2021. Municipal wastewater treatment using a membrane aerated biofilm reactor. *Journal of Environmental Engineering and Science,* 17**,** 99-107.

Wagner, B., Daigger, G. & Love, N. 2022. Assessing membrane aerated biofilm reactor configurations in mainstream anammox applications. *Water Science and Technology,* 85**,** 943-960.

Wang, J., Liang, J., Sun, L., Shen, J. & Wang, M. 2021. Achieving reliable partial nitrification and anammox process using polyvinyl alcohol gel beads to treat low-strength ammonia wastewater. *Bioresource technology,* 324**,** 124669.

Werner, J. J., Koren, O., Hugenholtz, P., Desantis, T. Z., Walters, W. A., Caporaso, J. G., Angenent, L. T., Knight, R. & Ley, R. E. 2012. Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys. *The ISME journal,* 6**,** 94-103.

Wu, G. & Yin, Q. 2020. Microbial niche nexus sustaining biological wastewater treatment. *npj Clean Water,* 3**,** 33.

Wu, Y., Wu, Z., Chu, H., Li, J., Ngo, H. 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.

Yin, Q., Sun, Y., Li, B., Feng, Z. & Wu, G. 2022. The r/K selection theory and its application in biological wastewater treatment processes. *Science of the Total Environment***,** 153836.

Zhang, B., Yu, Q., Yan, G., Zhu, H., Xu, X. Y. & Zhu, L. 2018. Seasonal bacterial community succession in four typical wastewater treatment plants: correlations between core microbes and process performance. *Scientific reports,* 8**,** 4566.

Zhou, Y., Li, R., Guo, B., Xia, S., Liu, Y. & Rittmann, B. E. 2022. The influent COD/N ratio controlled the linear alkylbenzene sulfonate biodegradation and extracellular polymeric substances accumulation in an oxygen-based membrane biofilm reactor. *Journal of hazardous materials,* 422**,** 126862.

Zhou, Y., Li, R., Guo, B., Zhang, L., Zou, X., Xia, S. & Liu, Y. 2020. Greywater treatment using an oxygen-based membrane biofilm reactor: Formation of dynamic multifunctional biofilm for organics and nitrogen removal. *Chemical Engineering Journal,* 386**,** 123989.

Chapter 5 – Establishing Stable Nitritation in MABR Through Aeration Control

5.1. Introduction

Biological removal of nitrogen from municipal wastewater (MWW) is typically through the conventional nitrification and denitrification (ND) pathway. However, three major issues abound with this pathway; high energy demand for nitrification, external organic carbon requirement for denitrification for low C/N ratio wastewater and the potential release of nitrous oxide (N_2O), a greenhouse gas with >300 times greater potency than CO_2 (Liu, Ngo et al. 2019). An extensively researched alternative to the aforementioned pathway is partial nitrification-denitrification (PND) (nitritation). PND is derived from the fact that nitrite, the intermediary product between nitrification and denitrification processes can be directly reduced to nitrogen gas under anoxic condition. However, the capacity to provide conducive environment for AOB to strive, while inhibiting NOB is the key to establishing PND within a biological system. Studies have shown that the differential growth kinetics of AOBs and NOBs can be manipulated through careful control of process parameters including: system's DO, free ammonium (FA), free nitrite acid (FNA), temperature, pH, solid retention time (SRT) *etc*., to facilitate the out-selection of NOBs in most biological systems (Liu, Yang et al. 2017, Wang, Liang et al. 2021). For instance, low dissolved oxygen condition can inhibit NOB activities, while remaining harmless to AOBs due to the difference in AOB and NOB oxygen half-saturation constants and oxygen affinities (Ma, Domingo-Felez et al. 2017). The recently commercialized energy and cost-efficient MABR is a rapidly expanding technology with potential for nitrogen removal through the PND pathway. [3] Low dissolved oxygen

[3] A version of this chapter was accepted for publication in Journal of Environmental Engineering in October, 2023 as: S Ukaigwe, Y Zhang, K Lee, Y Liu" Establishing Stable Nitritation in MABR through Aeration Control".

condition has specifically been demonstrated to suppress NOB in MABR (Ma, Domingo-Felez et al. 2017, Bunse, Orschler et al. 2020, Chen, Cao et al. 2022, Ma, Piscedda et al. 2022). Moreover, the DO within the MABR can easily be regulated via manipulating air supply (Terada, Yamamoto et al. 2006, Park, Chung et al. 2015, Bunse, Orschler et al. 2020, Chen and Zhou 2021). However, to sustain NOB suppression in a low-oxygen habitat, intermittent aeration is required, as the oscillation between aerobic/anoxic phases will result in alternating aerobic (low DO) and anoxic operating conditions, which will control NOB adaptation to low DO condition (Wett, Omari et al. 2013). This study examines the feasibility of achieving and maintaining stable PND with high performance in MABR for mainstream wastewater treatment by regulating reactor DO through intermittent aeration. The focus is nitrite accumulation through controlled aeration and optimizing the aerated/non-aerated cycles.

5.2. Materials and methods

5.2.1. Reactor characterization - oxygen demand

Amongst the key parameters for determining MABR performance are the oxygen transfer rate (OTR) and oxygen transfer efficiency (OTE) (Côté, Peeters et al. 2015, Houweling, Peeters et al. 2017). Because biofilm develops on the membrane surface, oxygen transfer capacity of the biofilm depends on both the process operational parameters and the membrane properties. Oxygen needs to diffuse into and through the biofilm for pollutant degradation reaction to occur. However, biofilm surface area is not consistent, it changes as the biofilm thickness changes, thus MABR performance is expressed on the basis of the membrane surface area. According to Côté et al., (2015), OTR in MABR is directly proportional to the membrane surface area deployed (eqn.1).

$$OTR = J.S \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where:

OTR = oxygen transfer rate (g O_2/d)

S = membrane surface area (m^2)

J = oxygen flux through membranes (determined from experimental data)

$$J = \frac{24M_0}{V_m}\left(Q_{PF}.X_F - Q_{PE}.X_E\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where:

J = oxygen flux (g O_2/d/m^2)

M_O = molecular weight of oxygen (32 g/mol)

Q_{PF}, Q_{PE} = process gas feed and exhaust specific flow rates ($Nm^3/h/m^2$)

V_m = standard gas volume at STP (0.0224 m^3/mol)

X_F, X_E = molar fraction of oxygen in feed and exhaust gas

Table 5.1. Synthetic Wastewater Composition

Chem. Form	**NaOAc**	**C3H5NaO2**	**NH4Cl**	**K2HPO4**	**KH_2PO_4**	**$CaCl_2$. $2H_2O$**	**$MgSO_4$. $7H_2O$**	**$FeSO_4$ $7H_2O$**	**Micro nutrient soln.**
Value	938	210	191	30	25	15	12.5	10	0.5
Unit	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mL/L

Table 5.2. Influent Characteristics

Parameter	**TCOD**	**NH_3**	**TP**	**TN**
Value	520 ± 30	52 ± 3	10 ± 1	160 ± 3
Unit	(mg/L)	(mg/L)	(mg/L)	(mg/L)

Table 5.3. Reactor Operating Condition

Parameter	**Working volume**	**Mem. pore size**	**Mem. surface area**	**Air flow rate**	**Rec. rate**	**Influent Feed flow Rate**	**Press.**	**pH**
Value	80	0.1	0.042	45 -100	200	0.2	2	7.61 - 8.46
Unit	mL	μm	m^2	mL/min	mL/min	mL/min	Psi	

Table 5.4. Aeration Strategy

Aeration Mode	Aeration cycle (min)	Operational Time (day)	HR (hr.)	NH_4^+-N SLR (g/m^2 - day)	COD SLR (g/m^2 - day)	DO (mg/L)	OTR (gO^2/d)
Continuous	-	00 - 33	2.5	1.00 ± 0.07	9.6 ± 0.9	1.4 ± 0.8	9.28
Intermittent	5 on/5 off	35 - 58	2.5	1.04 ± 0.04	10.8 ± 0.3	0.3 ± 0.01	6.21
Intermittent	10 on/10 off	60 - 94	2.5	1.06 ± 0.32	10.8 ± 1.0	0.3 ± 0.01	6.01
Intermittent	10 on/15 on	96 - 114	2.5	1.00 ± 0.03	9.9 ± 0.3	0.2 ± 0.03	5.98

5.2.2. Experimental procedure

This study was carried out over a period of ~4 months using synthetic medium-strength municipal wastewater in an MABR with matured and stable biofilm. The operation was done in 4 consecutive phases. Phase (I) was performed under continuous aeration mode, while Phases (II-IV), were conducted under aerated and non-aerated cycles of 10, 20 and 25 minutes respectively. Each phase was operated until steady state was attained (at least three weeks) using the operating conditions outlined in Table 5.3. Steady-state was considered to have been attained when pollutants (COD and NH_4^+-N) removal efficiencies were within a 10% variation consecutively over two to three readings. The reactor was inoculated with returned activated sludge obtained from a full-scale biological nutrient removal wastewater treatment plant in Alberta. Influent flow was continuous at all operation conditions. Bulk liquid DO was continuously monitored and maintained between 0.2 – 0.6 mg/L. Prepared influent was stored at 4 °C and used within 1 week. The entire operation was done under room temperature (22 ± 0.3°C) condition with no pH control.

5.2.3. Performance evaluation and sample analysis

Pollutants removal efficiency (PRE), nitrite accumulation rate (NAR) and nitrate production rate (NPR) were measured as performance indicators. PRE was estimated from

equation (3), while. NAR and NPR were estimated from equations (4) and (5). Bulk liquid DO, aeration pressure and pH were monitored and controlled to maintain reactor conditions.

$$PRE(\%) = \left(\frac{C_{Inf} - C_{Eff}}{C_{Inf}}\right) * 100\% \quad \text{..... (3)}$$

$$NAR(\%) = \left(\frac{NO_2^- - N_{eff}}{NO_2^- - N_{eff} + NO_3^- - N_{eff}}\right) * 100\% \quad \text{..... (4)}$$

$$NPR(\%) = \left(\frac{NO_3^- - N_{eff} -- NO_3^- - N_{in-}}{NH_4^+ - N_{inf} + NH_4^+ - N_{eff}}\right) * 100\% \quad \text{..... (5)}$$

Where: *PRE = Pollutant removal efficiency*

NAR = Nitrite accumulation rate

NPR = Nitrate production rate

$C_{inf/eff}$ = Influent/Effluent pollutant concentration

$NO_2^- - N_{inf/eff}$ =influent/effluent nitrite nitrogen

$NO_3^- - N_{inf/eff}$ = influent/effluent nitrate nitrogen

$NH_4^+ - N_{inf/eff}$ = Influent /Effluent ammonia nitrogen

5.2.4. Deoxyribonucleic acid (DNA) extraction

Deoxyribonucleic acid (DNA) was extracted from the reactor inocula and biofilm samples collected during continuous aeration, intermittent aeration intervals of 5, 10 and 15 minutes at HRTs of 2.5 h, using a DNeasy PowerSoil Kit (Qiagen Inc., Toronto, Canada) and following the manufacturer's protocol. The purity and concentration of the DNA extracted from each sample were measured with NanoDrop One (ThermoFisher, Waltham, MA). Microbial communities in the samples were analyzed for the 16S ribosomal ribonucleic acid (rRNA) gene sequence. The sequence was amplified by the polymerase chain reaction (PCR) using primer sets with the sequencing adaptors 515 F (GTGCCAGCMGCCGCGG) and 806 R (GGACTACHVGGGTWTCTAAT)

(Apprill et al., 2015). DNA amplicon samples were stored at -20 °C, before being sent to the Génome Québec Innovation Centre (Montréal, QC, Canada) for barcoding and sequencing on the Illumina MiSeq PE250 platform using the primer pair 515F/806R. Forward and reverse reads of the raw sequence were paired and screened, and chimeras were removed with the "DADA2" algorithm in the QIIME2 pipeline (Callahan, McMurdie et al. 2016). Taxonomy was determined with 99 % similarity in the GreenGenes database, version 13_8 (McDonald, Price et al. 2012, Werner, Koren et al. 2012). Raw sequence data can be accessed from the National Centre for Biotechnology Information (NCBI) GenBank (Bio Project Accession number PRJNA1012507).

5.2.5. AOB and NOB activity tests

AOB and NOB activity tests were conducted at the at phases II and III (5on:5 off and 10on:10 off) phases on days 58 and 94, while the reactor performance was optimal. The tests were carried out as follow: 30 mL mixed liquor from the reactor was transferred to 160 mL serum bottles. The mixed liquor contained 50 mg/L NH_4^+-N and 350 mg $CaCO_3$/L for the AOB activity test, while 20 mg/L NO_2^--N, and 140 mg $CaCO_3$/L f– the NOB activity test. NH_4^+-N and NO_2-N concentrations in the substrate were determined based on the highest NH_4^+-N concentration and the highest accumulated NO_2-N concentration in a typical medium strength wastewater condition. The pH of the mixed liquor was adjusted to 7.5-7.8 before the serum bottles were sealed with rubber plugs and aluminium caps. The serum bottles were shaken at 160 rpm at 20 °C. Liquid samples were then taken from the test bottle at 15, 30, 45, and 60 min and each time filtered immediately through 0.45 µm filters before NH_4^+-N (AOB activity) and NO_2^--N (NOB activity) measurents were done.

The highest AOB activity (ammonia oxidation rate) was calculated based on the slope of the linear regression curve of NH_4^+-N reduction versus time within 1 hour. While the highest NOB activity (nitrite oxidation rate) was determined based on the slope of the linear regression curve of NO_3-N production versus time during the 1hour test.

5.2.6. Statistical analysis.

Statistical analysis was performed to evaluate the MABR treatment performance. Analysis of variance (ANOVA) was carried out using Microsoft Excel® software. Correlations were considered statistically significant at the 95% confidence interval ($p < 0.05$).

5.3. Result and Discussion

5.3.1. Organic carbon removal

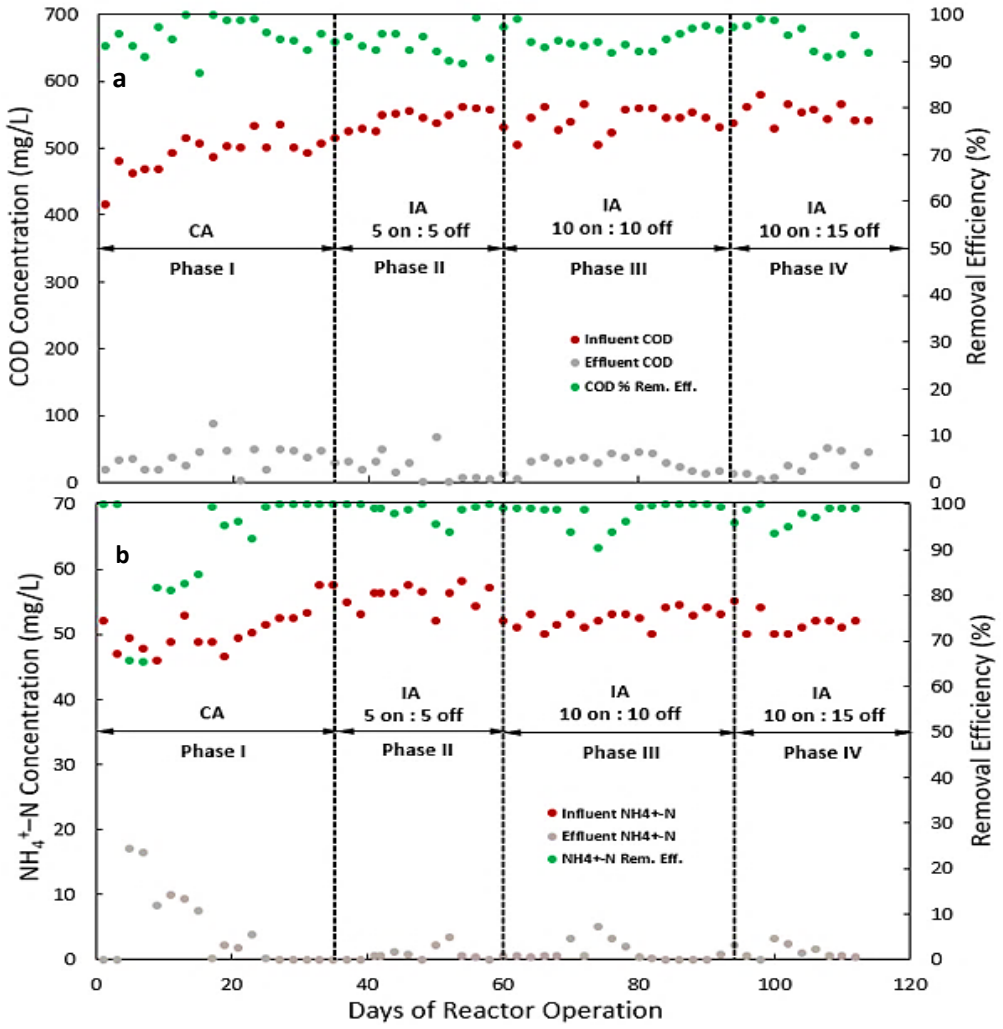


Figure 5.1a and b: Influent and effluent concentrations and removal efficiency profiles for a) COD at continuous and intermittent aerations cycles of 10 minutes, (5 on:5 off), 20 minutes (10 on:10 off) and 25 minutes (10 on:15 off). b) NH_4^+-N at continuous and intermittent aeration cycles of 10 minutes, (5 on:5 off), 20 minutes (10 on:10 off) and 25 minutes (10 on:15 off) at HRT of 2.5h.

Effluent COD concentration was consistently low at an average of 28 mg/L ± 18, at mean influent COD concentration of 520 ± 30 mg COD/L over the reactor operation time (Figure 5.1a). Mean removal efficiency was constantly > 90 % even as the reactor DO was gradually reduced through the extension of the cycle times (Table 5.3). This may have resulted because the influent COD consisted of easily biodegradable acetate (Ge, Wang et al. 2015). High COD removal at low HRT (1.4 -12h) and low DO (0.91 - 0.32mg/L) has been reported by other MABR studies (Zhou, Li et al. 2020, Ukaigwe, Zhou et al. 2021, Zhang, Jiang et al. 2022).

Average COD removal efficiency decreased by 3 % from 97 to 94 % following a switch in aeration pattern from continuous (Phase I) to a 10-minute cycle (5 minutes on, 5 minutes off) (Phase II). A decrease in reactor DO, which reduced microbial activities maybe a plausible reason for this drop in performance. Under aerobic conditions, DO is an critical limiting factor for microbial activities (Bian, Wu et al. 2022).

After 34 days, the operation cycle was increased to 20 minutes (10 minutes on, 10 minutes off) (Phase III), followed by 25 minutes (10 minutes on, 15 minutes off (Phase IV), but the reactor maintained average COD removal efficiency >90 %. This is most likely because COD consumption occur in multiple locations; aerobic, anoxic and anaerobic zones due to the reactor's stable and stratified biofilm that contained diverse microbial communities. Zhou et al., (2020) reported that matured MABR biofilm maintained autotrophic and heterotrophic microbes at DO concentration ranging between 0.37- 1.67 mg/L. High COD removal efficiency has frequently been reported for MABR with matured and stable biofilm (Côté, Peeters et al. 2015, Suarez, Piculell et al. 2019, Sanchez-Huerta, Fortunato et al. 2022, Wang, Liu et al. 2022).

Overall, COD removal efficiency did not significantly depreciate ($p > 0.05$) over the course of study for all the conditions tested.

5.3.2. Ammonia removal

The consistently low effluent NH_4^+-N concentration of 3 ± 2.5 mg/L at a mean influent concentration of 52 ± 3 mg/L shows good performance at all operation conditions (Figure 5.1b). Phase I (continuous aeration), the reactor maintained a high NH_4^+-N removal efficiency of ~99 %, after stabilization (Days 25-35), which was due to the aerobic condition (DO > 0.5) (Table 5.3) with abundant and active nitrifying microbes within the MABR system. High NH_4^+-N removal efficiency has frequently been reported for MABR with matured biofilm (Houweling, Peeters et al. 2017, He, Wagner et al. 2021, Ravishankar, Nemeth et al. 2022, Zhang, Jiang et al. 2022).

As the aeration pattern was changed to intermittent of 5 minutes on, 5 minutes off (Phase II), average ammonia removal remained stable at 98%. With further adjustments of the operation cycle to 20 minutes (10 minutes on, 10 minutes off) (Phase III), the NH_4^+-N removal efficiency initially declined to 94 % (Days 70 -78) before returning to 98%. The initial decline and subsequent rebound may be attributable to the sensitivity of nitrifiers to the conditions within the biofilm (Ge, Wang et al. 2015). Ma et al., (2017), applied experimental and model studies to underscore the strong impact of biofilm DO dynamics on NH_4^+-N oxidation efficiency.

Upon maintaining high and stable performance (98%) for 14 days (Days 80-94), the cycle time was increased to 25 minutes (Phase IV). At this condition, NH_4^+-N removal efficiency remained unchanged at 98 %, thus the system can be assumed to have attained steady state. The result from this study is consistent with the results from the simulation studies of Ma et al., (2017) and Pellicer-Nàcher et al., (2010), that predicted decrease in performance upon changing aeration pattern from continuous to intermittent. However, the decline observed in this study was insignificant ($p > 0.05$).

The consistently high NH_4^+-N removal efficiency obtained in this study may be attributed to the presence of residue DO within the biofilm and activities of aerobic bacteria that adapted to

low DO concentration within the reactor. High adaptive capacities in response to variabilities in environmental and operational conditions have often been reported for nitrifiers (Awolusi, Kumari and Bux 2015, Latocheski, da Rocha and Braga 2022).

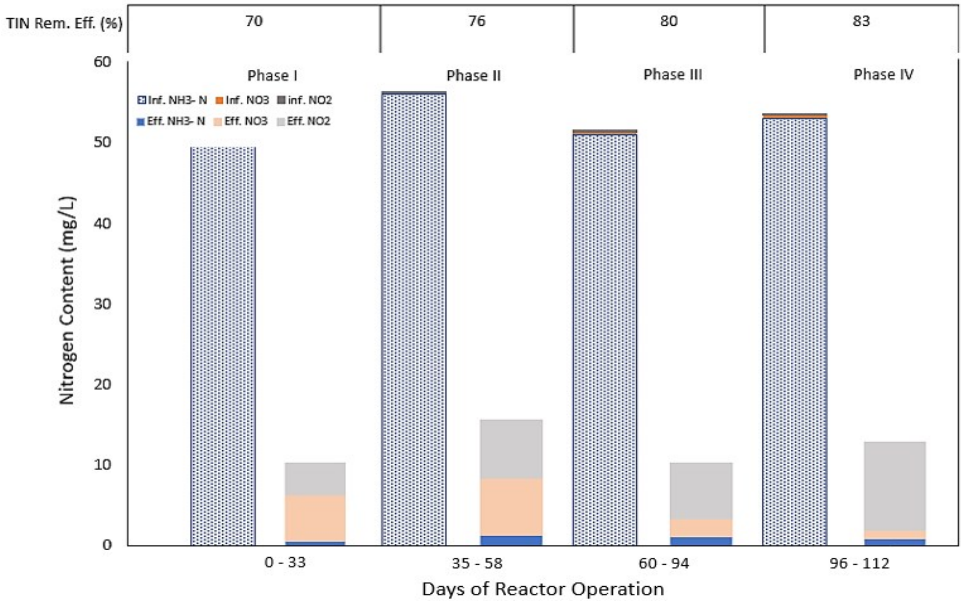


Figure 5.2: Profile of the change in the nitrogen contents within the MABR and total inorganic nitrogen removal efficiency resulting from continuous and intermittent aeration over the duration of the study.

5.3.3. Inorganic nitrogen removal

The average TIN removal attained in Phase I was 70 ± 6 %. From Phases II to IV, TIN removal efficiency increased from 76 to 83 % (Figure 5.2). The increase in TIN removal efficiency may have resulted from improved denitrification from organic carbon availability and from extended anoxic periods as a result of intermittent aeration which decreased the bulk DO to anoxic/anaerobic levels (Table 5.3). This result is in agreement with the findings of Bunse et al., (2020) who reported improvement in total nitrogen removal from 23 - 69% as a result of improved denitrification from intermittent aeration. Furthermore, Li et al., (2022) reported decreasing MABR biofilm DO through intermittent aeration enhanced partial denitrification and thus improved total nitrogen removal efficiency.

Figure 5.2 also show the concentrations of inorganic nitrogen content; NH_4^+-N, NO_2^--N, and NO_3^--N observed in the influent and effluent of the MABR. During the continuous aeration mode, NO_3^--N was dominant in reactor effluent (compared to NO_2^--N), but consistently decreased from a mean value of 5.7 to 0.94 mg/L as the aeration pattern was switched from continuous to intermittent (Phase II – IV). Which implies that NO_2^--N oxidation declined considerably. This is consistent with the work of other investigators who reported significant NO_3^--N lag after switching aeration mode from continuous to intermittent (Pellicer-Nàcher, Sun et al. 2010, Gilbert, Agrawal et al. 2014, Miao, Zhang et al. 2016, Ma, Piscedda et al. 2022). Average effluent NO_2-N concentration increased by > 75 % from 3.80 mg/L to 6.78 mg/L, as the aeration mode was switched from continuous (Phase I) to 10 minutes intermittent aeration cycle (5 on:5 off) (Phase II), which agrees with the result of Downing and Nerenberg, (2008) and others, who reported nitrite accumulation in an intermittently aerated MABR under anoxic bulk liquid condition.

As the operation stabilized, the aerobic/anoxic intervals were extended by 100 %, (10 on:10 off) (Phase III), but the system maintained its stability, and NO_2-N concentration remained constant. This maybe be attributed to "nitrite loop" effect as suggested by Aqeel et al., (2019), a situation that develops in a biofilm whereby nitrite is oxidized by NOB to nitrate, followed by reduction back to nitrite by denitrifying bacteria (Aqeel, Weissbrodt et al. 2019). Further extension of the aerated and non-aerated cycle to 25 minutes (Phase IV), led to an increase in effluent NO_2^--N concentration to 10.2mg/L. NO_2^--N accumulation may largely be attributed to the NOB suppression, and consequently nitrogen removal was dominantly via nitritation.

5.3.4. Microbial activity evaluation

At the onset of intermittent aeration mode (5 minutes on; 5 minutes off) (Phase II). AOB activity was 4.53 mgN/hr.gVSS (Figure 5.3). More than 30 days later, after the extension of aerated and non-aerated cycle to 20 minutes (10 on; 10 off), AOB activity had increased to 12.6 mgN/hr.gVSS (Phases III), indicating the decrease in system DO concentration did not inhibit AOB activities, rather, the activities were enhanced. AOB are generally less affected by the feast/famine conditions associated with cyclic aeration (Pellicer-Nàcher, Franck et al. 2014).

In contrast, NOB activity declined by > 60 % from 1.17 mgN/hr.gVSS to 0.46 mgN/hr.gVSS within the same time frame, which signifies that the decrease in system DO notably inhibited NOB activities. Miao et al (2016) reported > 80% decrease in NOB activities and an increase in AOB activities following a change in aeration strategy from continuous to intermittent in the treatment of low-ammonium sewage. Pellicer-Nàcher et al., (2010), also reported suppression of NOB activities in an intermittently aerated MABR.

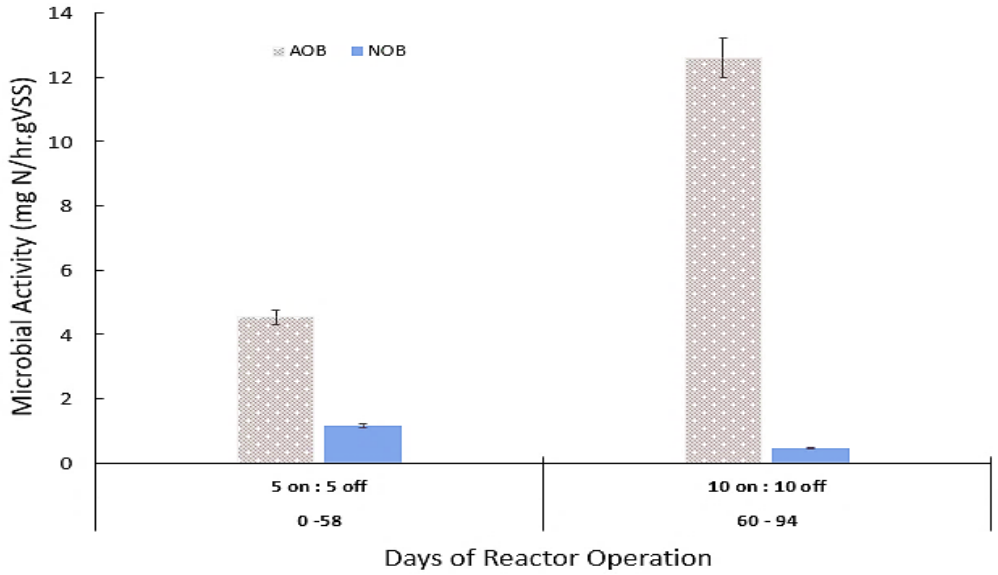


Figure 5.3: Profile of AOB and NOB activities within the MABR at 5 minutes on, 5 minutes off (5 on:5 off) and 10 minutes on, 10 minutes off (10 on: 10 off) aerated and non-aerated time intervals.

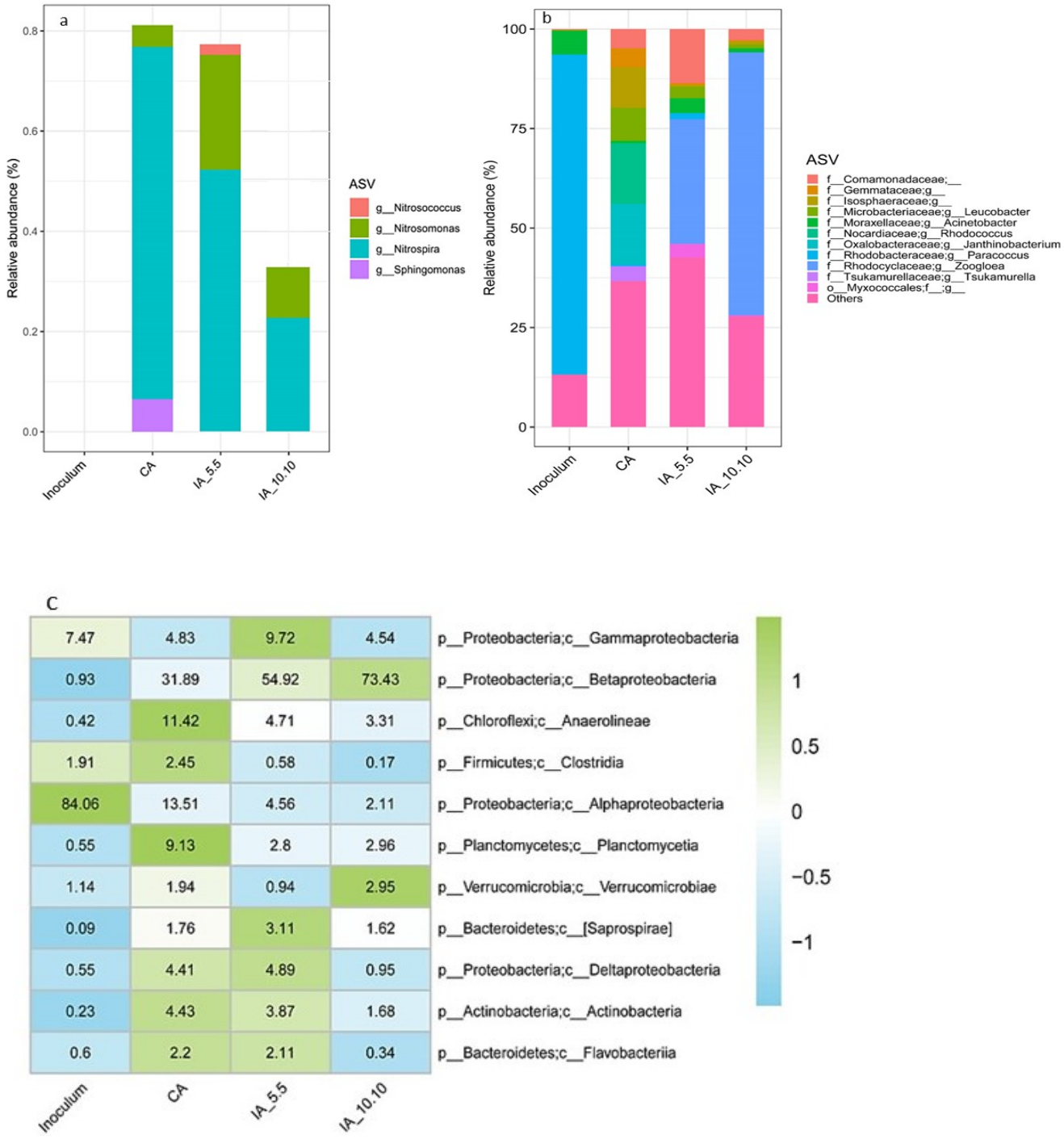


Figure 5.4a, b and c: a) High-throughput sequencing of 16S rRNA gene amplicons showing the distribution of the biofilm microbial community at different aeration intervals (CA; 5 on: 5off, and 10 on:10 off). Ammonia-oxidizing bacteria (AOB) and Nitrite-oxidizing bacteria (NOB) at genus level. b) The most abundant amplicon sequence variants (ASVs) at family and genus levels. c) The most abundant amplicon sequence variants (ASVs) at phyla and class level classification.

5.3.5. Microbial community transition

Change in operating conditions frequently affect microbial community, as a subpopulation of a group may lyse when conditions are become unfavourable (Aqeel, Weissbrodt et al. 2019). In this study 16S rRNA sequencing was used to evaluate the microbial community dynamics over time. Figure 5.4a, b and c show resilient community of nitrifiers (AOBs and NOBs), aerobic denitrifiers (ADB), and anaerobic denitrifiers (AnDB) that adapted to varying operation conditions. The microbial diversity changed from the inoculum with lower diversity to an increase in diversity over time during the continuous aeration mode, this transition may be due to high substrate limitation in the inoculum prior to use. But, 20 days after the aeration condition was changed from continuous to intermittent (5 on:5 off), microbial diversity decreased. Which may have resulted from the disruption of the microbial living conditions.

However, as the system environ transitioned to more anoxic condition due to longer non-aeration periods (10 on:10 off), the microbial diversity increased. Increased microbial diversity may be attributed to longer reactor operation time, that allowed slow-growing microbes to emerge. Additionally, intermittent aeration stimulated the development of anaerobic region and anaerobes within the biofilm. The change in diversity is evident at the phylum level, where Proteobacteria decreased between continuous and intermittent aeration modes (Phase I and II), before eventually increasing. Bacteroidetes, Actinobacteria and Firmicute phyla that play critical roles in pollutant removal within the biofilm (Zhou, Li et al. 2020, Ukaigwe, Zhou et al. 2021, Iorhemen, Ukaigwe et al. 2022) all followed similar trend (Figure 5c).

At class level, important communities involved in pollutant removal from MWW found in high RA within this system include: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Actinobacteria, and Planctomycetia, (Tian, Hui et al. 2021). The dominant amplicon sequence variants (ASVs) in the microbial community, that

displayed distinct shift in microbial diversity are shown in Figure 5.4b. The dominant ASVs in the inoculum which increased over time in at all three operating conditions are from the genera: *Acinetobacter*, *Paracoccus* and *Planctomycete*. In contrast, ASVs of, *Dokdonella*, *Leucobacter* and *Prosthecobacter* decreased. This observation is consistent with the findings of Pellicer-Nàcher et al., (2014) who reported significant shifts in the microbial community after switching the aeration pattern of an MABR from continuous to intermittent.

Other genera found within this system that are of importance in organic carbon and nitrogen removal from wastewater through nitrification and denitrification include *Dechloromonas, Zoogloea, Thauera, Simplicispira, Hydrogenophaga, Pseudomonas,* and *Nitrosomonas* (Pathak, Wang and Janka 2022, Wang, Liu et al. 2022). The high relative abundance of ADBs including: *Acinetobacter*, *Pseudomonas, Zoogloea, Dechloromonas* and AnDBs including: *Rhodobacter, Thauera, Paracoccus etc.*, present within the biofilm confirms the existence of stable and stratified layers within the intermittently aerated biofilm.

5.3.6. Correlation between operating conditions and key nitrifiers in the biofilm

Oxygen-limiting conditions, generally result in low activities for AOB and NOB, however, they are capable of maintaining their growth under anoxic conditions relying on limited DO concentration (Sliekers, Haaijer et al. 2005, Spieck, Keuter et al. 2014). A comparison of the observable shift in nitrifiers RA and diversity within the biofilm for the three operating conditions is shown in Figure 5.4a. Three members of the AOB community were present within the biofilm; *Sphingomonas, Nitrosococcus* and *Nitrosomonas* (Mota, Ridenoure et al. 2005, Fitzgerald, Camejo et al. 2015, Zhou, Li et al. 2020) with *Nitrosomonas* as the dominant population. *Nitrosomonas* were present at all operating conditions, mostly due to the availability of stimulating conditions for AOB activity and high adaptability. Chen et al., (2022), reported the presence of *Nitrosomonas* at all operation condition in an intermittently aerated MABR treating nitrogenous wastewater.

On the other hand, *Nitrosococcus* was below detection limit prior to the intermittent aeration phase. 20 days after the system was switched to intermittent aeration, they became detectable, but decreased afterwards. However, this *Nitrosococcus* belongs to the *betaproteobacteria* genus *Nitrosomonas. Sphingomonas* was only detectable at the continuous aeration phase at high DO concentration. With a shift in DO concentration, it declined beyond detection, which is non-typical of *Sphingomonas* due to their high adaptive capacities, however according to the report of Yim et al., (2010) different strains of *Sphingomonas* responds differently to operational conditions (Yim, Yau et al. 2010).

In contrast, *Nitrospira* was the sole NOB genus present at all conditions during this phase of the study because they have the capacity to outcompetes other NOBs at low DO and low substrate concentrations (Schramm, De Beer et al. 2000). *Nitrospira* displayed relatively higher relative abundance during the continuous aeration condition. Upon switching to intermittent aeration 20 days later, *Nitrospira* decreased by ~3% and further decreased by ~50% 40 days later at a longer aeration interval of 10 on:10 off. This is in agreement with several studies that reported switching from continuous to intermittent aeration diminished reactor DO, placed NOB at a disadvantage and limited their activities (Gilbert, Agrawal et al. 2014, Pellicer-Nàcher, Franck et al. 2014, Li, Li et al. 2022). Moreover, under oxygen-limited condition, AOB releases hydroxylamine (HAO) and nitric oxide (NO), both of which inhibit NOB (Kostera, Youngblut et al. 2008). However, under these conditions NOBs had larger relative abundance than AOBs. This could have resulted because *as* k-strategists with high substrate affinity, they proliferate at limited substrate concentrations and strong competitive environment (Koch, Lücker et al. 2015, Sharif Shourjeh, Kowal et al. 2021, Zhao, Guo et al. 2022). Moreover, long SRTs like that obtainable in MABR, support *Nitrospira* proliferation (Wang, Terada et al. 2009). A couple of studies have also reported larger NOBs populations (Regmi, Miller et al. 2014, Choi, Cho et al. 2018, Liu, Chen et al. 2019). Choi et al.,

(2018) and Liu et al., (2019) both reported *Nitrospira* as dominant nitrifier under hypoxia and low ammonium concentration conditions. Thus, intermittent aeration significantly decreased the relative abundance of *Nitrospira,* but did not completely suppress it within the aeration intervals applied.

5.4. Conclusion

In this study, an MABR was operated with continuous and intermittent aeration modes and limited DO condition (0.2 – 0.3mg/L) in order to initiate and maintain stable nitritation for municipal wastewater treatment. Results obtained shows that switching the process air supply mode from continuous to intermittent, switched the system from nitratating to nitritating. Although the reactor could not successfully handle 25 minutes of aerated ad non-aerated cycle (10 on: 15 off), but with 20 minutes (10 on: 10 off), NPR was significantly reduced to 1%, >95% of the influent ammonium nitrogen was removed while NAR and TIN removal efficiency increased above 90 and 80% respectively for >100 days of MABR operation. Microbial activity analysis showed that the operating condition applied significantly reduced NOB activities without impacting AOB activities.

In general, the MABR displayed promising results for nitrogen removal from MWW through nitritation under mainstream conditions.

5.5. References

Akinnawo, S. O. (2023). "Eutrophication: Causes, Consequences, Physical, Chemical and Biological Techniques for Mitigation Strategies." Environmental Challenges: 100733.

APHA AWWA, W. (1998). "Standard methods for the examination of water and wastewater 20th edition." American Pub[li]c Health Association, American Water Work Association, Water Environment Federation, Washington, DC.

Aqeel, H., D. G. Weissbrodt, M. Cerruti, G. M. Wolfaardt, B.-M. Wilén and S. N. Liss (2019). "Drivers of bioaggregation from flocs to biofilms and granular sludge." Environmental Science: Water Research & Technology **5**(12): 2072-2089.

Awolusi, O., S. Kumari and F. Bux (2015). "Ecophysiology of nitrifying communities in membrane bioreactors." International Journal of Environmental Science and Technology **12**: 747-762.

Bian, X., Y. Wu, J. Li, M. Yin, D. Li, H. Pei, S. Chang and W. Guo (2022). "Effect of dissolved oxygen on high C/N wastewater treatment in moving bed biofilm reactors based on heterotrophic nitrification and aerobic denitrification: Nitrogen removal performance and potential mechanisms." Bioresource Technology **365**: 128147.

Bunse, P., L. Orschler, S. Agrawal and S. Lackner (2020). "Membrane aerated biofilm reactors for mainstream partial nitritation/anammox: Experiences using real municipal wastewater." Water Research X **9**: 100066.

Chen, R., S. Cao, L. Zhang and Y. Zhou (2022). "NOB suppression strategies in a mainstream membrane aerated biofilm reactor under exceptionally low lumen pressure." Chemosphere **290**: 133386.

Chen, R. and Y. Zhou (2021). "Measure microbial activity driven oxygen transfer in membrane aerated biofilm reactor from supply side." Environmental Research **195**: 110845.

Choi, M., K. Cho, D. Jeong, Y.-C. Chung, J. Park, S. Lee and H. Bae (2018). "Effects of the ammonium loading rate on nitrite-oxidizing activity during nitrification at a high dose of inorganic carbon." Journal of Environmental Science and Health, Part A **53**(8): 708-717.

Côté, P., J. Peeters, N. Adams, Y. Hong, Z. Long and J. Ireland (2015). A new membrane-aerated biofilm reactor for low energy wastewater treatment: pilot results. WEFTEC 2015, Water Environment Federation.

Downing, L. S. and R. Nerenberg (2008). "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.

Fitzgerald, C. M., P. Camejo, J. Z. Oshlag and D. R. Noguera (2015). "Ammonia-oxidizing microbial communities in reactors with efficient nitrification at low-dissolved oxygen." Water research **70**: 38-51.

Ge, S., S. Wang, X. Yang, S. Qiu, B. Li and Y. Peng (2015). "Detection of nitrifiers and evaluation of partial nitrification for wastewater treatment: A review." Chemosphere **140**: 85-98.

Gilbert, E. M., S. Agrawal, F. Brunner, T. Schwartz, H. Horn and S. Lackner (2014). "Response of different Nitrospira species to anoxic periods depends on operational DO." Environmental science & technology **48**(5): 2934-2941.

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

He, H., B. M. Wagner, A. L. Carlson, C. Yang and G. T. Daigger (2021). "Recent progress using membrane aerated biofilm reactors for wastewater treatment." Water Science and Technology **84**(9): 2131-2157.

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

Houweling, D., J. Peeters, P. Cote, Z. Long and N. Adams (2017). "Proving membrane aerated biofilm reactor (MABR) performance and reliability: Results from four pilots and a full-scale plant." Proceedings of the Water Environment Federation **2017**(16): 272-284.

Iorhemen, O. T., S. Ukaigwe, H. Dang and Y. Liu (2022). "Phosphorus Removal from Aerobic Granular Sludge: Proliferation of Polyphosphate-Accumulating Organisms (PAOs) under Different Feeding Strategies." Processes **10**(7): 1399.

Koch, H., S. Lücker, M. Albertsen, K. Kitzinger, C. Herbold, E. Spieck, P. H. Nielsen, M. Wagner and H. Daims (2015). "Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus Nitrospira." Proceedings of the national academy of sciences **112**(36): 11371-11376.

Kostera, J., M. D. Youngblut, J. M. Slosarczyk and A. A. Pacheco (2008). "Kinetic and product distribution analysis of NO· reductase activity in Nitrosomonas europaea hydroxylamine oxidoreductase." JBIC Journal of Biological Inorganic Chemistry **13**: 1073-1083.

Lackner, S., A. Terada, H. Horn, M. Henze and B. F. Smets (2010). "Nitritation performance in membrane-aerated biofilm reactors differs from conventional biofilm systems." Water Research **44**(20): 6073-6084.

Latocheski, E. C., M. C. V. da Rocha and M. C. B. Braga (2022). "Nitrospira in wastewater treatment: applications, opportunities and research gaps." Reviews in Environmental Science and Bio/Technology **21**(4): 905-930.

Li, M., Y. Li, N. Wang, T. Li, H. Guo, Z. Wu, P. Zhang, B. Wang and B. Li (2022). "Achieving efficient nitrogen removal in a single-stage partial nitrification-anammox-partial denitrification (PN/A/PD) membrane aerated biofilm reactor (MABR)." Journal of Water Process Engineering **49**: 103100.

Liu, W., W. Chen, D. Yang and Y. Shen (2019). "Functional and compositional characteristics of nitrifiers reveal the failure of achieving mainstream nitritation under limited oxygen or ammonia conditions." Bioresource technology **275**: 272-279.

Liu, W., Q. Yang, B. Ma, J. Li, L. Ma, S. Wang and Y. Peng (2017). "Rapid achievement of nitritation using aerobic starvation." Environmental Science & Technology **51**(7): 4001-4008.

Liu, X., M. Kim, G. Nakhla, M. Andalib and Y. Fang (2020). "Partial nitrification-reactor configurations, and operational conditions: Performance analysis." Journal of Environmental Chemical Engineering **8**(4): 103984.

Liu, Y., H. H. Ngo, W. Guo, L. Peng, D. Wang and B. Ni (2019). "The roles of free ammonia (FA) in biological wastewater treatment processes: A review." Environment international **123**: 10-19.

Ma, Y., C. Domingo-Felez, B. G. Plósz and B. F. Smets (2017). "Intermittent aeration suppresses nitrite-oxidizing bacteria in membrane-aerated biofilms: a model-based explanation." Environmental science & technology **51**(11): 6146-6155.

Ma, Y., A. Piscedda, A. D. L. C. Veras, C. Domingo-Félez and B. F. Smets (2022). "Intermittent aeration to regulate microbial activities in membrane-aerated biofilm reactors: Energy-efficient nitrogen removal and low nitrous oxide emission." Chemical Engineering Journal **433**: 133630.

McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight and P. Hugenholtz (2012). "An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea." The ISME journal **6**(3): 610-618.

Mehrabi, S., D. Houweling and M. Dagnew (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.

Miao, Y., L. Zhang, Y. Yang, Y. Peng, B. Li, S. Wang and Q. Zhang (2016). "Start-up of single-stage partial nitrification-anammox process treating low-strength swage and its restoration from nitrate accumulation." Bioresource Technology **218**: 771-779.

Mota, C., J. Ridenoure, J. Cheng and F. L. de los Reyes III (2005). "High levels of nitrifying bacteria in intermittently aerated reactors treating high ammonia wastewater." FEMS Microbiology Ecology **54**(3): 391-400.

Park, S., W. Bae and B. E. Rittmann (2010). "Operational boundaries for nitrite accumulation in nitrification based on minimum/maximum substrate concentrations that include effects of oxygen limitation, pH, and free ammonia and free nitrous acid inhibition." Environmental science & technology **44**(1): 335-342.

Park, S., J. Chung, B. E. Rittmann and W. Bae (2015). "Nitrite accumulation from simultaneous free ‐ ammonia and free ‐ nitrous ‐ acid inhibition and oxygen limitation in a continuous ‐ flow biofilm reactor." Biotechnology and Bioengineering **112**(1): 43-52.

Pathak, S., S. Wang and E. Janka (2022). "Achieving Partial Nitritation in Anammox Start-Up Environment." Water **14**(2): 229.

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

Peng, Y. and G. Zhu (2006). "Biological nitrogen removal with nitrification and denitrification via nitrite pathway." Applied microbiology and biotechnology **73**: 15-26.

Preisner, M. (2020). "Surface water pollution by untreated municipal wastewater discharge due to a sewer failure." Environmental Processes **7**(3): 767-780.

Preisner, M., E. Neverova-Dziopak and Z. Kowalewski (2021). "Mitigation of eutrophication caused by wastewater discharge: A simulation-based approach." Ambio **50**(2): 413-424.

Ravishankar, H., A. Nemeth, G. Massons, D. Puig, D. Zardoya, N. Carpi, P. N. Lens and B. Heffernan (2022). "Factors impacting simultaneous nitrification and denitrification in a membrane aerated biofilm reactor (MABR) system treating municipal wastewater." Journal of Environmental Chemical Engineering **10**(5): 108120.

Regmi, P., M. W. Miller, B. Holgate, R. Bunce, H. Park, K. Chandran, B. Wett, S. Murthy and C. B. Bott (2014). "Control of aeration, aerobic SRT and COD input for mainstream nitritation/denitritation." Water research **57**: 162-171.

Sanchez-Huerta, C., L. Fortunato, T. Leiknes and P.-Y. Hong (2022). "Influence of biofilm thickness on the removal of thirteen different organic micropollutants via a Membrane Aerated Biofilm Reactor (MABR)." Journal of Hazardous Materials **432**: 128698.

Schramm, A., D. De Beer, A. Gieseke and R. Amann (2000). "Microenvironments and distribution of nitrifying bacteria in a membrane‐bound biofilm." Environmental microbiology **2**(6): 680-686.

Sharif Shourjeh, M., P. Kowal, X. Lu, L. Xie and J. Drewnowski (2021). "Development of strategies for AOB and NOB competition supported by mathematical modeling in terms of successful deammonification implementation for energy-efficient WWTPs." Processes **9**(3): 562.

Sliekers, A. O., S. C. Haaijer, M. H. Stafsnes, J. G. Kuenen and M. S. Jetten (2005). "Competition and coexistence of aerobic ammonium-and nitrite-oxidizing bacteria at low oxygen concentrations." Applied microbiology and biotechnology **68**: 808-817.

Spieck, E., S. Keuter, T. Wenzel, E. Bock and W. Ludwig (2014). "Characterization of a new marine nitrite oxidizing bacterium, Nitrospina watsonii sp. nov., a member of the newly proposed phylum "Nitrospinae"." Systematic and applied microbiology **37**(3): 170-176.

Suarez, C., M. Piculell, O. Modin, S. Langenheder, F. Persson and M. Hermansson (2019). "Thickness determines microbial community structure and function in nitrifying biofilms via deterministic assembly." Scientific reports **9**(1): 1-10.

Terada, A., T. Yamamoto, R. Igarashi, S. Tsuneda and A. Hirata (2006). "Feasibility of a membrane-aerated biofilm reactor to achieve controllable nitrification." Biochemical engineering journal **28**(2): 123-130.

Tian, H., M. Hui, P. Pan, J. Huang, L. Chen and J. Zhao (2021). "Performance and microbial ecology of biofilms adhering on aerated membrane with distinctive conditions for the treatment of domestic sewage." Environmental Technology **42**(3): 459-467.

Ukaigwe, S., Y. Zhou, M. Shaheen and Y. Liu (2021). "Municipal wastewater treatment using a membrane aerated biofilm reactor." Journal of Environmental Engineering and Science **17**(2): 99-107.

Wang, B., S. Liu, X. Yao, Y. Liu and G. Qi (2022). "Alternating aeration strategy to reduce aeration energy demand for aerobic granular sludge and analysis of microbial community dynamics." Environmental Science: Water Research & Technology **8**(5): 1111-1125.

Wang, H., G. Xu, Z. Qiu, Y. Zhou and Y. Liu (2019). "NOB suppression in pilot-scale mainstream nitritation-denitritation system coupled with MBR for municipal wastewater treatment." Chemosphere **216**: 633-639.

Wang, J., J. Liang, L. Sun, J. Shen and M. Wang (2021). "Achieving reliable partial nitrification and anammox process using polyvinyl alcohol gel beads to treat low-strength ammonia wastewater." Bioresource technology **324**: 124669.

Wang, R., A. Terada, S. Lackner, B. F. Smets, M. Henze, S. Xia and J. Zhao (2009). "Nitritation performance and biofilm development of co-and counter-diffusion biofilm reactors: modeling and experimental comparison." Water research **43**(10): 2699-2709.

Werner, J. J., O. Koren, P. Hugenholtz, T. Z. DeSantis, W. A. Walters, J. G. Caporaso, L. T. Angenent, R. Knight and R. E. Ley (2012). "Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys." The ISME journal **6**(1): 94-103.

Wett, B., A. Omari, S. Podmirseg, M. Han, O. Akintayo, M. Gómez Brandón, S. Murthy, C. Bott, M. Hell and I. Takács (2013). "Going for mainstream deammonification from bench to full scale for maximized resource efficiency." Water science and technology **68**(2): 283-289.

Yang, Q., Y. Peng, X. Liu, W. Zeng, T. Mino and H. Satoh (2007). "Nitrogen removal via nitrite from municipal wastewater at low temperatures using real-time control to optimize nitrifying communities." Environmental science & technology **41**(23): 8159-8164.

Yim, M.-S., Y. C. Yau, A. Matlow, J.-S. So, J. Zou, C. A. Flemming, H. Schraft and K. T. Leung (2010). "A novel selective growth medium-PCR assay to isolate and detect Sphingomonas in environmental samples." Journal of microbiological methods **82**(1): 19-27.

Zhang, L.-Q., X. Jiang, H.-W. Rong, C.-H. Wei, M. Luo, W.-C. Ma and H.-Y. Ng (2022). "Exploring the carbon and nitrogen removal capacity of a membrane aerated biofilm reactor for

low-strength municipal wastewater treatment." Environmental Science: Water Research & Technology **8**(2): 280-289.

Zhao, H., Y. Guo, Q. Wang, Z. Zhang, C. Wu, M. Gao and F. Liu (2022). "The Summary of Nitritation Process in Mainstream Wastewater Treatment." Sustainability **14**(24): 16453.

Zhou, Y., R. Li, B. Guo, L. Zhang, X. Zou, S. Xia and Y. Liu (2020). "Greywater treatment using an oxygen-based membrane biofilm reactor: Formation of dynamic multifunctional biofilm for organics and nitrogen removal." Chemical Engineering Journal **386**: 123989.

Chapter 6 – Exploring the Impact of Combined Inocula on MABR Biofilm Development Dynamics

6.1. Introduction

MABR is amongst the next generation energy-efficient biological wastewater treatment technologies with potential for additional energy optimization. Studies on MABR application have reported improvements on all performance indices (Pérez-Calleja et al., 2022, Ma et al., 2022, Peeters et al., 2017). However, like all biofilm systems, MABR biofilm thickness exerts significant influence on its performance (Martin & Nerenberg, 2012; Sanchez-Huerta, et al., 2022). Unchecked biofilm growth leads to excessive biofilm thickness, which reduces biofilm operational surface area, inhibits mass transfer, promotes sloughing, and ultimately results in poor performance (Boltz and Daigger 2010, Shoji, Itoh et al. 2020).

Inoculum origin and properties may impact MABR biofilm thickness because they determine initial microbial activities and community heterogeneity (Chen, Lan et al. 2017)Li et al., 2016; Pellicer-Nàcher *et al*., 2010). MABRs are traditionally inoculated with conventional activated sludge (CAS) typically dominated by a group of phylogenetically and physiologically distinct aerobic bacteria and low biomass concentration. However, research suggests that microbial community structure may be distinct for different wastewater compositions and operating conditions (Zhang, Guo et al. 2015, Agrawal, Karst et al. 2017).

Aerobic granular sludge (AGS) is another novel wastewater treatment technology with energy-efficiency potentials. AGS harbour phylogenetically different microbial groups and high EPS content (Nancharaiah and Sarvajith 2019). EPS properties are crucial to biofilm development and performance. Moreover, the inclusion of AGS granules to biofilm systems has been suggested to be beneficial, because it can provide additional attachment surface for biofilm, while its continuous physical abrasion that may regulate biofilm thickness (Krause, Zimmermann et al.

2010). Thus, the inoculation of an MABR with CAS and AGS sludge may diversify the microbial community and impact the biomass properties.

In this study, we explored the effect of combined CAS and AGS sludge on the biofilm properties of an MABR operated without biofilm control for the treatment of high-strength synthetic wastewater to assess the impacts of mixed-inocula on the biofilm properties, biofilm thickness control and performance. The hypothesis is that mixed-inocula could contain microbial population of higher biodiversity while continuous recirculation of rigid AGS granules will produce shear with significant cumulative influence to regulate biofilm growth.

6.2. Materials and Methods

6.2.1. Influent and inoculum sludge properties

The synthetic wastewater used in this study had the following composition: NaOAc 938 mg/L, $C_3H_5NaO_2$ 210 mg/L, NH_4Cl 191mg/L, K_2HPO_4 30 mg/L, KH_2PO_4 25 mg/L, $CaCl_2.2H_2O$ 15 mg/L, $MgSO_4.7H_2O$ 12.5 mg/L, $FeSO_4.7H_2O$ 10 mg/L, 0.5 mL/L micronutrient solution, as described in (Tay, Liu and Liu 2002, Syron, Semmens and Casey 2015). The micronutrient solution contained: H_3BO_4 0.05 g/L, $ZnCl_2$ 0.05 g/L, $CuCl_2$ 0.03 g/L, $MnSO_4.H_2O$ 0.05 g/L, $(NH_4)_6$ Mo_7O_{24} $4H_2O$ 0.05 g/L, $AlCl_3$ 0.05 g/L, $CoCl_2$ $6H_2O$ 0.05 g/L, $NiCl_2$ 0.05 g/L. This gave averages of 554 ± 224 mg/L and 261 ± 178 mg/L for COD and ammonia concentrations respectively. Two inocula in the ratio of 1:2 was used in this study; returned activated sludge taken from a local municipal wastewater treatment plant in Alberta and the AGS sludge was taken from a lab reactor that had been in operation for >50 days, with well-developed compact and rigid granules. The returned activated sludge had TSS and VSS of 7.630 ± 0.01 and 6.104 ± 0.01 g/L respectively, while the AGS sludge has TSS and VSS of 7.21 ± 0.02 and 5.768 ± 0.016 g/L.

6.2.2. Experimental procedure

This study was performed over a period of ~8 months. The initialization period including reactor stabilization and process parameter adjustments took about 1 month. Thereafter, the reactor was operated continuously for 7 months, using the parameters determined from the initial operation conditions. Start-up HRT was 24h, the MABR was operated at this HRT for about 40 days before being sequentially reduced to a final value of 4h over a 6-month period. Each HRT represented a stage, and each stage was operated until steady-state is attained (at least three weeks), using the operating conditions outlined in Table 6.1. Steady-state was considered to have been attained when pollutants (COD and $NH4^{+}$-N) removal efficiencies were within a 10% variation consecutively over two to three readings (4 - 6 days). The entire operation was done in 4 continuous stages with synthetic wastewater at room temperature (22 ± 0.3°C). 1.0 M $NaHCO_3$ was used to adjust influent pH to 8.2 ± 0.4. The returned activated sludge was acclimated to the substrate for 5 d in a batch mode before being used to start the MABR**.** Influent and air were continuously supplied at all operation conditions. Prepared influent was stored at 4 °C and used within 1 week. No form of conventional biofilm thickness control was employed throughout the study period. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analysis were conducted to observe the amount of biomass that had accumulated on the membrane surface over the study period. Furthermore, biofilm properties (density and thickness), EPS contents and the MABR performance potential were evaluated at the end of the study.

Table 6.1. Summary of the Operating Parameters

Parameter	Value
NH_3 concentration (mg/L)	261 ± 178
COD (mg/L)	554 ± 224
NH_4^+ loading rate (g/m^2 - d)	2.2 ± 1.84
COD loading rate (g/m^2 - d)	5.00 ± 2.6
Lumen Pressure (psi) (13.8 kpa)	1.8 - 2.2
Influent type (synthetic)	High strength wastewater
Inoculating sludge	CAS + AGS sludge

Table 6.2. Characteristics of CAS and AGS Sludges [a]

Parameter (mg/L)	V_S	V_{AGS}	Unit
TCOD	4100 ± 25.6	7300 ± 30.5	mg/L
SCOD	1275 ± 8.7	1832 ± 3.8	mg/L
NH_4^+- N	192 ± 2.5	375 ± 1.92	mg/L
NO_2 - N	1.14 ± 0.02	1.63 ± 0.–1	mg/L
NO_3 - N	5.93 ± 0.26	6.91 ± 0.–2	mg/L
TN	1028 ± 4.2	537 ± 1.5	mg/L

[a] = V_S – value of inoculum sludge; V_{AGS} – value of AGS sludge

6.2.3. Density and thickness analysis

Biofilm density evaluation was complemented with thickness measurement. For areal density determination, two representative membranes with biofilm were detached from the membrane bundle and dried overnight in an oven at 105 ^oC. The dried samples were weighed and the biofilm was mechanically removed from the membrane as described by (Hu, Xu et al. 2013) and the membranes were reweighed. Biofilm density was defined as the difference in weight between

the membrane with biofilm and the membrane without biofilm, with the dry biomass normalized to the growth area of the wet biofilm for the calculation of biomass per unit area of membrane. Biofilm mass was calculated based on the total number of membranes in the reactor, while biofilm thickness was measured using a digital Vernier caliper.

6.2.4. Extracellular polymeric substances (EPS) component extraction

To extract EPS from biofilm, one membrane with a length of approximately 200 mm was detached from the membrane bundle and the biofilm on the membrane surface was removed using the method described by (Luo, Chen et al. 2015). The detached biofilms were centrifuged at 3000 g (Thermo Sorvall Lynx 4000, Centrifuge, USA) at 21± 0.3 ˚C) for 5 min and the supernatant was removed. Approximately half of the biofilm sludge was used to determine the volatile suspended solids (VSS). VSS were measured according to standard methods (APHA AWWA 1998). The remaining half of the biofilm sludge was mixed with 20 mL phosphate buffered saline (PBS), followed by 15 min of vortex mixing (VWR® Vortex Mixer, Fixed Cup Head, Avantor, USA) at room temperature. The mixture was treated by ultrasound (FS30H Ultrasonic cleaner, Fisher Scientific, PA, Mexico) for 3 min and incubated in a water bath (Elmasonic E30H, Germany) at 80 ˚C for 20 min. The treated biofilm sludge was centrifuged for 15 min, at 10,000 g (Thermo Sorvall Lynx 4000, Centrifuge, USA) and the supernatant was filtered using 0.45 μm membrane filters. Filtrate containing EPS was purified in a dialysis membrane (Spectrum™ Labs Spectra/Por™) for 24 h. Polysaccharide and protein contents of the filtrate were determined using the phenol-sulfuric acid method with glucose as the standard (Frølund, Palmgren et al. 1996) and the Coomassie brilliant blue G-250 dye-binding method using bovine serum albumin as the standard (Pierce and Suelter 1977).

6.2.5. Cells viability analysis

Biofilm samples were obtained from the MABR membrane, stained with 1 mg/mL propidium iodide solution (LIVE/DEAD Backlight Bacterial Viability Kits) using 100 μL SYTO9 (Molecular Probes), and incubated in the dark at room temperature for 20 min. The biofilm samples were washed with phosphate buffered saline (PBS) after staining. The distribution of viable and nonviable cells was observed in the biofilm using fluorescence microscopy (Leica DM750 Fluorescence Microscope, PA, USA) at wavelengths of 488 nm and 515 nm, which correspond, respectively, to the excitation wavelengths of the SYTO9 and the propidium iodide contained in the staining reagent.

6.2.6. SEM characterization

For SEM and TEM characterization, a single representative membrane was detached from the membrane bundle and carefully cut with a sterile surgical scalpel this is to ensure that the original biofilm structures are preserved. Samples were cut into approximately 5 x10 mm piece each, from different parts of the membrane (top, middle, and end) in order to increase the sample representativeness. Additionally, the samples were then treated as listed in Table 6.3.

6.2.7. Deoxyribonucleic acid (DNA) extraction

DNA was extracted from the reactor inocula and from biofilm samples collected at HRTs of 24, 10, 6, and 4 h, respectively, using a Dneasy PowerSoil Kit (Qiagen Inc., Toronto, Canada) and following the manufacturer's protocol. The purity and concentration of the DNA extracted from each sample were measured with NanoDrop One (ThermoFisher, Waltham, MA). DNA amplicon samples were stored at -20 °C, before being sent to the Génome Québec Innovation Centre (Montréal, QC, Canada) for barcoding and sequencing on the Illumina MiSeq PE250 platform using the primer pair 515F/806R. Forward and reverse reads of the raw sequence were paired and screened, and chimeras were removed with the "DADA2" algorithm in the QIIME2 pipeline

(Callahan, McMurdie et al. 2016). Taxonomy was determined with 99% similarity in the GreenGenes database, version 13_8 (McDonald, Price et al. 2012, Werner, Koren et al. 2012).

6.2.8. Statistical analysis.

Statistical analysis was performed to evaluate the biofilm performance at each HRT. Analysis of variance (ANOVA) was carried out using Microsoft Excel® software. Correlations were considered statistically significant at the 95% confidence interval ($p < 0.05$).

6.3. Results and Discussion

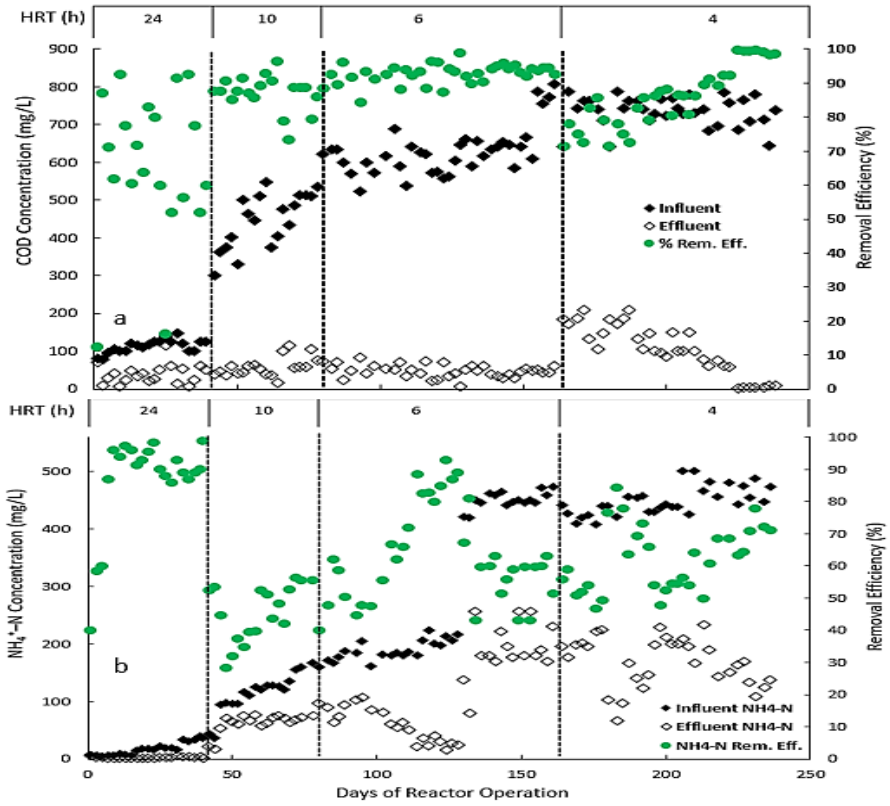


Figure 6.1a and b. Pollutant removal potential of the MABR at the different HRTs of the study period. (a) COD removal efficiency; (b) NH_4^+-N removal efficiency

6.3.1. Organic carbon removal

The MABR maintained an increasingly high organic carbon removal efficiency for the first 3 stages of the study, that is HRT 24 -6h (Figure 6.1a). For the first 40 days (HRT of 24h), at an average influent COD of 112 ± 17mg/L, which is equivalent to a load of 0.6 ± 0.2 gCOD/d/m^2, the mean COD removal efficiency was 66 ± 21%. At this condition, mean effluent COD concentration was 36 ± 26 mg/l. However, as the as HRT was gradually decreased to 10 and 6h respectively (days 42 – 161), the average load on the system increased to 4.8 ± 0.9 gCOD/d/m^2, while the removal efficiency steadily increased to 93 ± 3 %. This may be due to an increasing biomass concentration with increased microbial community and ease of sodium acetate biodegradation by heterotrophic and aerobic microorganisms (Houweling & Daigger, 2019). Additionally, MABR has been reported to be highly efficient in COD removal for most wastewater types (Sanchez-Huerta, Fortunato et al. 2022, Sun, Li et al. 2022). Mean effluent COD concentration on the other hand increased but became more stable at 48 ± 17 mg/l, despite the increased influent COD concentration, this may imply a steady biofilm performance under shock loading condition.

At HRT of 4h, average COD removal efficiency dropped to 84%. The decrease of COD removal efficiency at this HRT may be attributed the higher load as reactor SLR almost doubled from average of 4.8 ± 0.9 - 8.1± 0.8 gCOD/d/m^2. Moreover, reports have confirmed that increased load on the reactor may lead to decrease in pollutant removal efficiency (Ukaigwe et al., 2021). However, this recorded decrease in performance was found to be statistically insignificant ($p>0.05$).

6.3.2. Ammonium-nitrogen removal

Four HRTs of 24, 10, 6 and 4h were applied in this study with mean influent $NH4^+$–N concentration ranging from of 6.4 ± 5 mg L^{-1} to 448 ± 24mg L^{-1} (Figure 6.1b). Within the first 40 days of operation at HRT of 24h, which is equivalent to a load of 0.03 ± 0.02 gNH_3/d/m^2. The MABR attained an average removal efficiency of 87 ± 10%. 36 days later (HRT =10h), the removal

efficiency decreased significantly ($p < 0.05$) to 46 ± 7% as the MABR was subjected to higher shock loads of 0.54 ± 0.35 $gNH_3/d/m^2$.

However, as process HRT was further reduced to 6h, which is 150% increase in load (1.4 ± 0.5 $gNH_3/d/m^2$), $NH4^+$–N removal efficiency increased to 63 ±12%, this increase in performance was most likely induced by increased biofilm thickness after more than 100 days of reactor operation. Thicker biofilms have been reported to have higher performance within their optimum thickness limit due to higher volume-to-surface area ratio and higher and stable microbial community (Suarez, Piculell et al. 2019, Sanchez-Huerta, Fortunato et al. 2022).

Further increase in reactor load to 4.6 ± 0.4 $gNH_3/d/m^2$ through HRT reduction to 4h did not destabilize the system as removal efficiency showed a narrow decrease in performance to 61.7 ±12% but then plateaued at this value. This confirms that stable biofilm had developed within the MABR. Moreover, the recirculation of high density AGS sludge within the reactor may have enabled the maintenance of the biofilm thickness through steady physical abrasion of the biofilms surface by the AGS sludge, which accelerated loose-biofilm detachment to create thin, but stable biofilm. Thin biofilm has been reported to enhance external mass transfer rates, thereby providing higher overall removal efficiency (Shoji, Itoh et al. 2020), however, their ammonia removal potential may be limited (Sanchez-Huerta, Fortunato et al. 2022).

Biofilm thickness was more consequential on $NH4^+$–N removal efficiency, than on organic carbon removal efficiency at same operating conditions. This maybe due to low microbial density from the thin biofilm. Multiple organisms are able to degrade organic carbon, whereas $NH4^+$–N oxidation is mainly performed by very specific group of bacteria (AOB and NOB)(Tian, Hui et al. 2021). Moreover, nitrification is inhibited at ammonia concentrations of ≥100 mg/L (Paśmionka, Bulski et al. 2021).

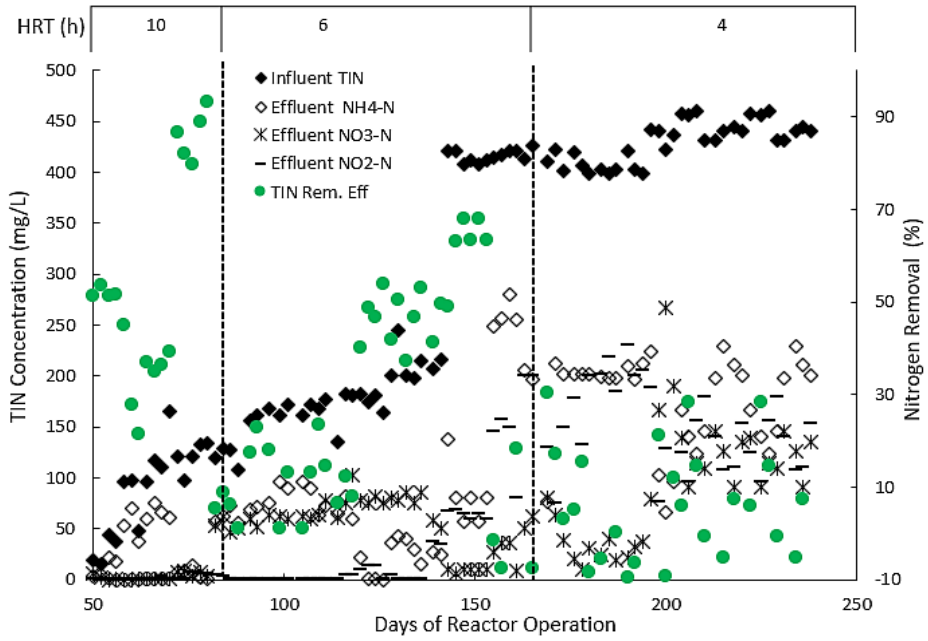


Figure 6.2. Total inorganic nitrogen removal efficiency at different HRTs.

6.3.3. Total inorganic nitrogen removal

Analysis of TIN removal performance was initiated at stage 2 of the study (HRT = 10h). The average TIN removal efficiency obtained at this stage was 55 ± 24%, with a mean influent TIN concentration of 74 ± 46mg/L. However, this declined to 32 ± 26% as the mean influent TIN concentration increased by more than 100% to 235 ± 100mg/L. Further increase of the influent TIN concentration to an average of 430 ± 20mg/L caused both significant decrease in removal efficiency to 3 ± 13 % and a destabilization of the system. This may have resulted from inhibition of nitrification and the thinness of the biofilm which hampered biofilm stratification and invariably the denitrification ability of the MABR (Shanahan and Semmens 2006). The ability to integrate nitrification and denitrification in a single stage is a major advantage of the MABR, simultaneous nitrification- denitrification (SND) improves TIN removal efficiency (Zhang, Jiang et al. 2022), however, this process is dependent amongst other factor on the thickness of the MABR biofilm. Minimum biofilm thickness for this process has been reported as 0.45mm (Paśmionka, Bulski et al.

2021, Wagner, Daigger and Love 2022). In addition, nitrifying and denitrifying populations must be present in sufficient quantities within the MABR biofilm to achieve SND (Ravishankar et al., 2022). Biomass limitation is a major challenge of thin biofilms, and can result in poor performance (He, Wagner et al. 2021, Lu, Bai et al. 2021, Tian, Hui et al. 2021). The thinness of the biofilm obtained in this study contributed to poor TIN removal performance. Ammonia conversion dynamics is shown in Figure 6.2.

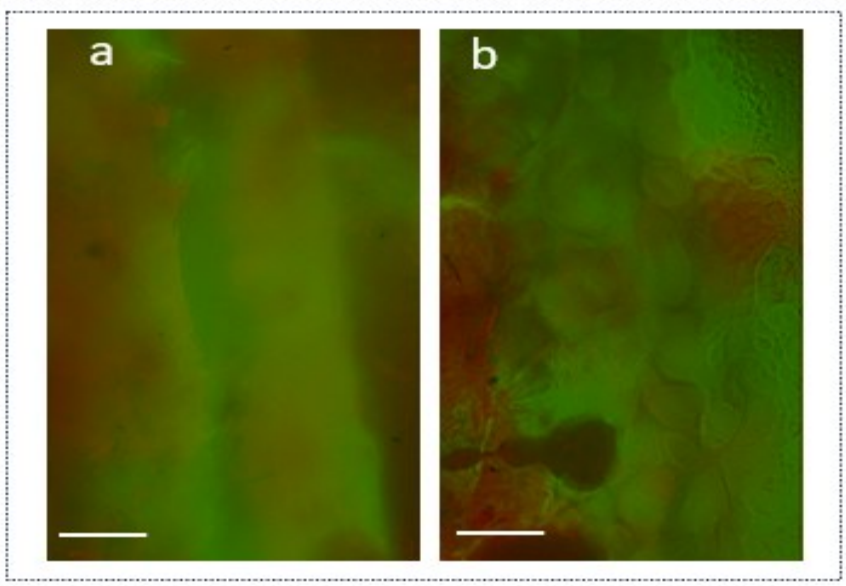


Figure 6.3. Fluorescence microscopy image showing the distribution of live (green) and dead (red) cells in the biofilm. Images were taken from two locations on day 230 at HRT of 4h. Image is at 200x magnification and 1mm scale.

Table 6.3. SEM and TEM Processing Procedures and Operating Condition

Steps	SEM	TEM
Day 1 **Fixation**	2.5% Glutaraldehyde 2% Paraformaldehyde in 0.1 M Phosphate Buffer (pH 7.2 – 7.4) for 24hrs	2.5% Glutaraldehye 2% Paraformaldehyde in 0.1M Phosphate Buffer (pH 7.2 – 7.4)
Washing	10 min × 3 times in PB 0.1M	10 – 15 min × 3 times in PB 0.1M
Post-fixation	1 % OSMIUM TETROXIDE in 0.1M Phosphate Buffer – 1 hour	1 % OSMIUM TETROXIDE in 0.1M Phosphate Buffer – 1 hour
Washing	10 min × 3 times in PB 0.1M	10 -15 min × 3 times in PB 0.1M
Dehydration **(Ascending ethanol series)**	30% ethanol - 100% 15’	50% ethanol – –00% 15-25’
Drying **(Ascending HMDS series)**	Ethanol: HMDS 75:25 - 25:75 15’ HMDS 15’	1:1–Ethanol: Spurr for 1-3 hours.
Day 2 **Pt Sputter coating/examination**	Mount on SEM stubs Sputter Coat with Au/Pd Examine with SEM (15 mA, 2 min)	N/A
Curing	N/A	Change to fresh pure Spurr resin Embed in flat molds with fresh Spurr Resin CURE – overnight in oven @ 70 ºC
Day 3 **Sectioning**	N/A	Sections blocks into 70 to 90 nm thicknesses
Staining of sections on grid	N/A	Stain with uranyl acetate, followed by lead citrate
Examination	N/A	Using Philips – FEI Model = Morgagni 268
Operating conditions	15–20 kV, high vacuum	80 kV

6.3.4. Viable cells analysis

Fluorescence microscopy reveals colonies with low number of viable cells in the biofilms (Figure 6.3), which may have resulted from high influent $NH4^{+}$–N concentration. High ammonium concentration invariably results in the presence of free ammonia (FA) under alkaline condition (Liu et al., 2019). FA has been reported as strong inhibitor to some key microorganisms in biological wastewater treatment (Park et al., 2015). A condition that could decrease specific microbial activities, reconfigure microbial community structure, break down the extracellular polymeric substances (EPS) and kill the living cells (Zhang et al., 2018), thereby decreasing viable cells within the system. Low viable cells may have contributed to the low (~60%) $NH4^{+}$–N removal efficiency obtained in this study, which is lower than the average 80% generally reported for MABR (Shaowei, Fenglin et al. 2008, Houweling, Peeters et al. 2017).

6.3.5. SEM analysis

Cross-sectional SEM micrograph of the biofilm reveals non-dense and non-homogenously distributed biofilm on the membrane surface, a spectrum of morphological diversity dominated by rod-shaped (bacillus), round (cocci) and curved-rod (comma) shape (vibrio) microbes. A few dormant spores, individually developing microbes, aggregates of microbes covered in self-produced extracellular matrix and spaces with sufficient surface area for more microbial colonization. This aligns with the low viable cells revealed by the viable and dead cells analysis. Biofilm as living cells can grow or shrink depending on process conditions. However, to realise SND, both the nitrifying and denitrifying population must be present in sufficient quantities in the MABR system. 16S rRNA sequencing was utilized to unveil the identities of the microbes within the biofilm.

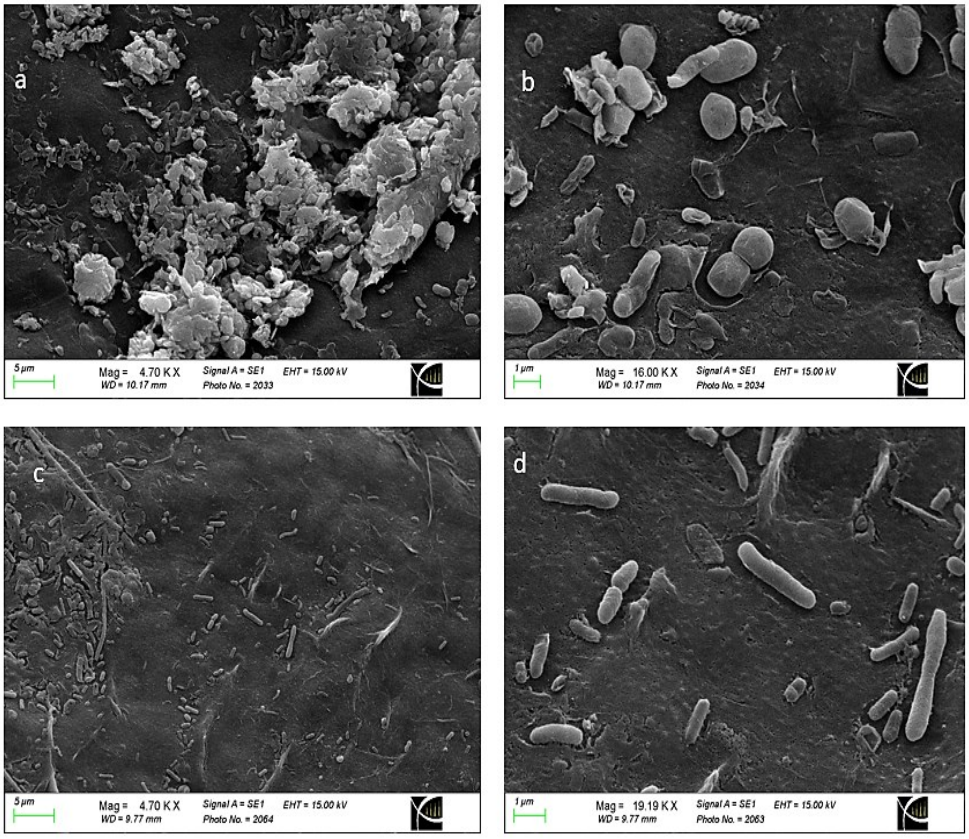


Figure 6.4. The surface morphology of the MABR biofilm profiles, visualized on a scanning electron microscope (SEM). Images are taken at random locations along the PVDF membrane surface at different magnification to cover large observation areas. Magnification ranged from 4.70 – 19.19k, (a - d).

6.3.6. TEM analysis

Examining the morphology and internal biofilm profile of biofilm using TEM reveals demarcation between the interstices and membrane boundaries and microbes firmly attached to the biofilm surfaces surrounded by glycocalyx (Figure 6.4c). Microbial cells appear sparse in most areas. This may have resulted from subdued biofilm growth from the multiple thickness control mechanism applied in this study. In addition, the sample preparation involved multiple washing, with multiple washing there is a possibility that part of the biofilm structure will be lost from the membrane surface during preparation (Walker et al., 2001). However, void layers in MABR biofilms have also been linked to protozoan predation. Predation impacts biofilm accumulation

(Aybar, M., 2019; Kim et al., 2020). Microbial spatial arrangement of cells within the glycocalyx are heterogenous, this is similar to the situation reported by Eighmy et al., (1983). Biofilm cells surrounded by EPS (grey) and inorganic deposits showing up as dark stains (Figure 6.4d) were also observed. Hu et al., (2013) reported similar findings on MBBR biofilm.

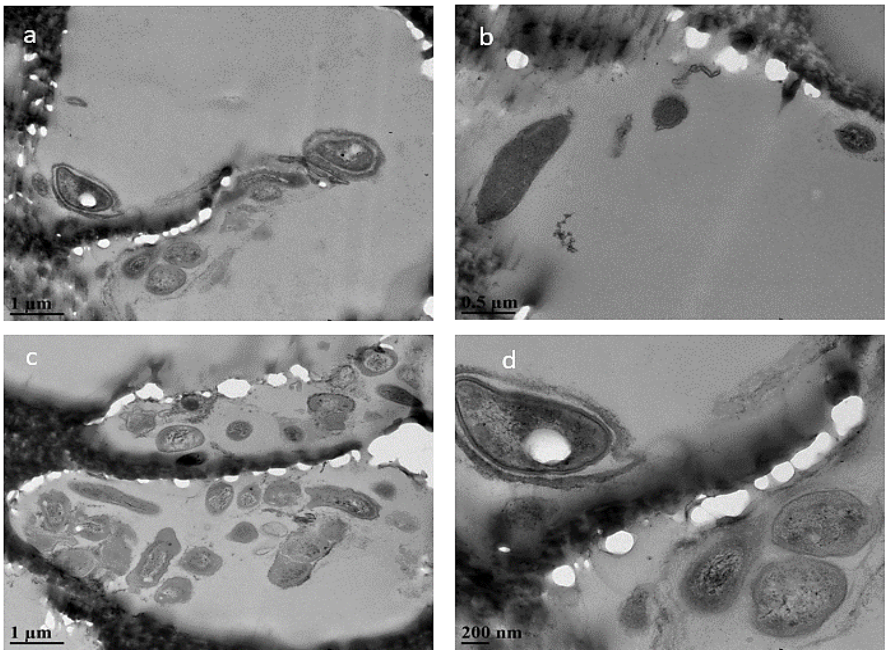


Figure 6.5. Transmission electron microscopic visualization of MABR biofilms profiles on PVDF membrane at different lengths of 1µm. (a and c) 0.5µm (b) and 200µm (d).

6.3.7. Biofilm thickness and density determination

Studies have demonstrated that active biofilm cleaning is required to prevent biofilm overgrowth during MABR operation (Shaowei, Fenglin et al. 2008, Stricker, Lossing et al. 2011). With the control strategy adopted in the study, in-process cleaning was not required in the >200 days of treating medium-strength municipal wastewater. Biofilm thickness from this study, was determined on day 230 to be 0.357 mm (Table 6.4), which fell below the minimum biofilm thickness

of 0.45 mm needed for biofilm stratification to accommodate SND within the biofilm (Wagner, Daigger and Love 2022). This value is also outside the range of 0.5 – 1mm that has been observed in most MABRs treating mainstream and high strength wastewaters (Terada, Hibiya et al. 2003, Pellicer-Nàcher, Sun et al. 2010, Gilmore, Terada et al. 2013, Sanchez-Huerta, Fortunato et al. 2022, Wagner, Daigger and Love 2022). Images of SEM and TEM showing sparse biomass concentration on membrane surface supports these results (Figure. 6.4 and 6.5).

On the other hand, the average biofilm density of 24.4g/L obtained falls within the range of 3 - 64g/L that has been reported for MABR biofilms (Shaowei, Fenglin et al. 2008, Pellicer-Nàcher and Smets 2014). The thinner than usual biofilm obtained may be due to subdued heterotrophic growth resulting from quick acetate degradation coupled with the high organic carbon removal potential of the AGS sludge (Wang, Liu et al. 2022). Moreover, Lin et al., (2015) reported that AGS sludge show amphiphilic character, therefore the presence of AGS in the reactor may interfere with capacity of the biofilm to adhere to the membrane surface. Surface hydrophobicity/hydrophilicity have been reported to have significant influence on biofilm initial attachment to membrane surfaces and biofilm growth during operation (Zhou, Kiely et al. 2021).

Most studies support positive correlation between thicker biofilm and hydrophobic surfaces as these surfaces are naturally more amenable to microbial attachment (Bhagwat et al., 2021). Others suggest that hydrophilic surfaces better promote biofilm adhesion (Hou, Li et al. 2013, Wu, Wu et al. 2019). But consensus on surface condition preference for biofilm attachment has so far not been reached as adhesiveness is also microorganism specific (De-la-Pinta, Cobos et al. 2019, Zhou, Kiely et al. 2021). Thinner and denser biofilms, though may have lower microbial diversity generally require less thickness control during operation which maybe an advantage in many MABR systems

where biofilm thickness control is a challenge. Additionally, biomass washout and system failure from sloughing do not occur with thin biofilms.

Table 6.4. Biofilm Thickness and Density Estimated from This Study

Component	Unit	Value
Average biofilm mass	g	2.389 ± 0.032
Weight differential	g	0.012 ± 0.001
Membrane length	m	0.220
Membrane outer diameter	m	0.0022
Membrane surface area	m^2	1.26
Membrane count	-	30
Average biofilm thickness	mm	0.357
Areal density	g/m^2	8.71 ± 0.4
Density	g/L	24.4 g/L ± 0.3

6.3.8. Inoculum choice assessment

One of the most important variables, which influences MABR performance, is the origin of the inoculum, because it determines the initial activity of the microbial community, microbial community heterogeneity, adaptive potentials and functions. (Li et al., 2016(Zhou, Wei et al. 2010, Chen, Lan et al. 2017). Inoculum origin becomes even more critical in systems like the MABR operated with infinite solids retention time (SRT), and no biomass withdrawal, conditions that mitigate drastic change of initial microbial community composition (Terada et al., 2010). In this study, the high number of heterotrophs in the inoculum, which remained throughout the study duration may have accelerated COD degradation, leading to the thin biofilm. Which signals that the inoculum may have influenced the biofilm properties.

6.3.9. EPS analysis

EPS is a major component of bioreactor biofilms that has been recognized to exert important influence on membranes surface. For instance, EPS could adsorb on the membrane surface making the surface more hydrophobic, thereby influencing biofilm growth on the membrane surface. This concept of membrane surface property modification by EPS has been supported by multiple investigations (De-la-Pinta, Cobos et al. 2019, Wu, Wu et al. 2019, Zhang, Feng et al. 2019). Moreover, since EPS compositions are mainly protein and polysaccharides with protein as the major fraction, more EPS content in sludge could infer high hydrophobicity (Mohan and Nagalakshmi 2020). Protein is reportedly responsible for inputting hydrophobicity on surfaces and promoting early stages of biofilm formation (Zhang, Guo et al. 2015, Mohan and Nagalakshmi 2020, Zhou, Kiely et al. 2021). Though, environmental factors and operational conditions can alter the EPS contents (Liang, Li et al. 2010, Zhang, Feng et al. 2019). EPS was extracted from the biofilms on day 230 at HRT of 4h.

The biomass concentration was determined to be 0.6gVSS/L, while the protein and polysaccharide concentrations were determined to be 33.8 and 7.34mg/L respectively, a PN/PS ratio of 4.5. A possible reason for the higher protein content could be the AGS sludge, which studies suggest have high EPS protein (Zhu, Zhou et al. 2015). Mass ratio of EPS content (PN/PS) has often been used as an indicator of hydrophobicity, with high PN/PS ratio indicating stronger hydrophobicity and more availability of adsorption sites (Zhang, Jia et al. 2018).

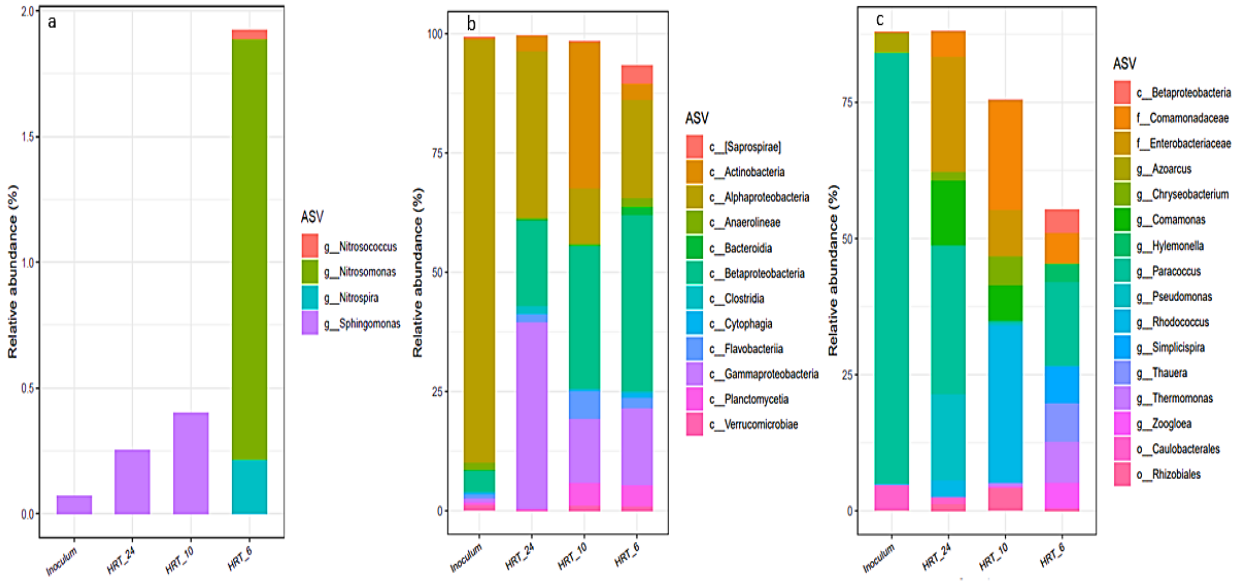


Figure 6.6. (a) High-throughput sequencing of 16S rRNA gene amplicons showing the distribution of the microbial community in the biofilm at HRTs of 24, 10, and 6 h. Ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) at genus level. (b) The most abundant amplicon sequence variants (ASVs) at HRTs of 24, 10, and 6 h at class level. (c) genus level classification.

6.3.10. Microbial community analysis

16S rRNA gene amplicons sequencing was used to identify the microbial community within the MABR. The inoculum had low microbial diversity, while the biofilm diversities generally increased over time. Predominant bacterial families present in the MABR included: Comamonadaceae (0.61-28.48%), Rhodobacteraceae (0.57 - 79.58%), Enterobacteriaceae (0.04 - 21.21%) and Pseudomonadaceae (0.01-15.74%) (Figure. 6.7). Comamonadeae, Xanthononadaceae, Pseudomonadeceae, Enterobacteriaceae and other bacterial families within the Proteobacteria phylum are biofilm formers. They are also involved in organic carbon oxidation and nutrient removal in wastewater (Adav, Lee and Lai 2010, Kelly, London et al. 2021, Iorhemen, Ukaigwe et al. 2022). The bacterial class with high relative abundance both in the inoculum and at all HTRs were Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Actinobacteria (Figure 6.6b). At the genus level, *Paracoccus, Pseudomonas, Comamonas*, *Thauera* and *Zoogloea* were

present in significant numbers (Figure 6.6c). *Zoogloea* genus is aerobic bacteria, capable of denitrification, while *Thauera* are primarily known for their anaerobic degradation. Both genera are common in WWTPs employing aerobic process (Begmatov, Dorofeev et al. 2022). Significant number of non-putative microbes were also found in the system (Figure 6.6b and c). Microbial relative abundance, changed over the course of the experiment, with some microbes detectable in the inoculum decreasing significantly, whereas others that were undetectable in the inoculum increased over time. This imply operational conditions impacted the each bacterial species differently (Yim, Yau et al. 2010). The microbial community biodiversity showed minimal changes over the course of the study (Figure 6.6b and c), which signified consistency in operational environment, most likely because the biofilm didn't have sufficient thickness for stratification.

6.3.10.1 Dynamics of nitrifying microbes

Nitrifiers generally have high adaptive capacities (Latocheski, da Rocha and Braga 2022), hence their presence in both inoculum and biofilm at all HRTs. At genus level, the relative abundance of nitrifiers in the MABR varied between HRTs. These include AOBs (*Nitrosomonas, Sphingomonas and Nitrosococcus*) and NOBs (*Nitrospira*) (Mota, Ridenoure et al. 2005, Krishna, Sathasivan and Ginige 2013, Fitzgerald, Camejo et al. 2015, Zhou, Li et al. 2020). The relative abundance of *Sphingomonas* though low in the inoculum, consistently increased between HRTs of 24 and 10h, this is most likely because nitrifiers are slow growers, and given conducive environment, they flourish. At an HRT of 6h, the relative abundance of *Sphingomonas* declined significantly. This may have been induced by the drop in the system DO as a result of increased load on the MABR. However, *Sphingomonas* reportedly have high adaptive capacity to extreme conditions (Awolusi, Kumari and Bux 2015), therefore the reason for the decline is unclear. But, studies report that different strains of *Sphingomonas* responds differently to operational conditions

(Yim, Yau et al. 2010). Nitrification is positively associated with *Sphingomonas* (Godzieba, Zubrowska-Sudol et al. 2022). *Sphingomonas* also have the ability to produce viscous polysaccharide that enhances biofilm formation and to decompose complex organic carbon (Koskinen, Ali-Vehmas et al. 2000, Czieborowski, Hübenthal et al. 2020, Kelly, London et al. 2021). MABR like other fixed-film systems have the capability to support the slow-growing *Nitrosomonas. Nitrosomonas population* became detectable at HRT of 6 h, and were relatively more abundant than the other nitrifiers (Figure 6.6a). Generally, AOBs are usually about twofold higher than the NOBs in wastewater treatment systems (Aqeel, Weissbrodt et al. 2019). In this study, the relative abundance of *Nitrosomonas* was ~6-fold higher than to the NOBs (*Nitrospira*) probably due to high ammonia concentration as *Nitrosomonas* always predominate in high NH_4^+-N concentration conditions (Terada, Sugawara et al. 2013). *Nitrosococcus* became detectable at HTR of 6, plausible explanation for this later appearance could be slower growth rates relative to heterotrophs, *Nitrosococcus* has been reclassified to *Nitrosomonas* (Braker and Conrad, 2011). In the same vein, *Nitrospira* became detectable at HRT of 6h with low relative abundance, due to slow-growth rates under limited substrate condition (Bunse, Orschler et al. 2020). Moreover, *Nitrospira* are generally more sensitive to ammonia concentration than other nitrifiers, which may have contributed to their low relative abundance (Latocheski, da Rocha and Braga 2022).

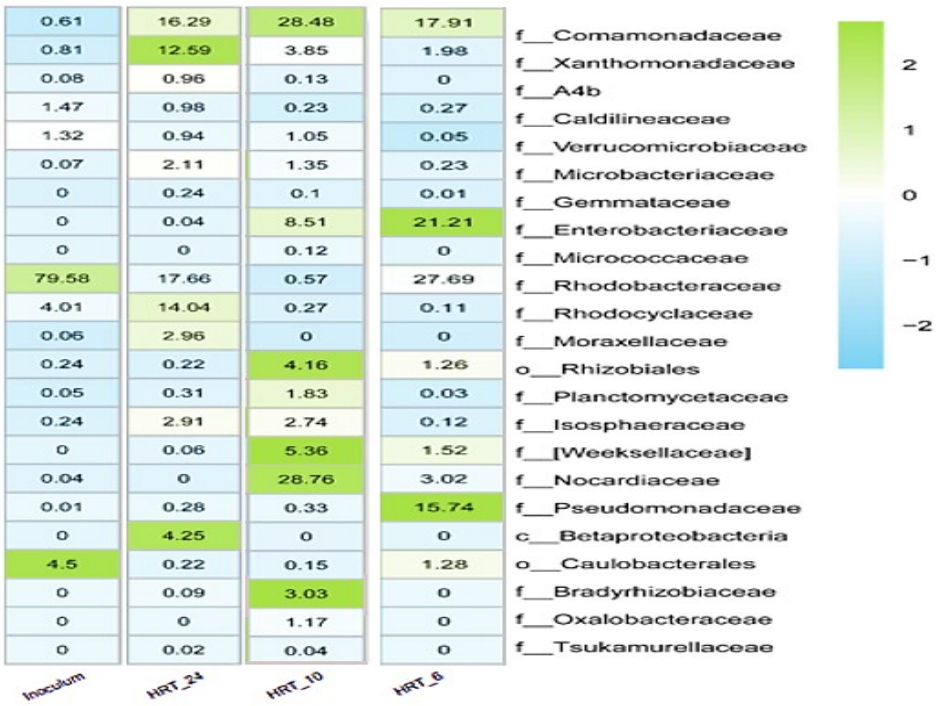


Figure 6.7. The 12 most abundant ASVs in the biofilm at HRTs of 24, 6 and 4 h at family level.

6.4. Conclusion

The effect of combined CAS and AGS sludge on MABR biofilm properties was explored. The MABR was operated without conventional biofilm control and was applied in the treatment of high strength synthetic wastewater to assess the impacts of mixed-inocula on MABR as a means of biofilm thickness control. End of the study, fluorescence microscopy revealed colonies with low number of viable cells in the biofilms, while SEM and TEM reveal non-dense and non-homogenously distributed biofilm on the membrane surfaces. Biofilm thickness on the other hand was 0.357mm which was lower than 0.5mm required for simultaneous nitrification-denitrification to occur within a biofilm. Thus, denitrification may have been inhibited. The effect of the biofilm thickness was more consequential on $NH4^{+}$–N and TIN removal than on COD removal efficiency. COD removal efficiencies were higher at all conditions studied, than $NH4^{+}$–N and TIN removal

efficiencies. Thus, the thickness control mechanisms applied may have inhibited biofilm growth instead of controlling overgrowth. More investigations are needed to corroborate and validate the results obtained in this study.

6.5. References

Adav, S. S., D. J. Lee and J. Y. Lai (2010). "Microbial community of acetate utilizing denitrifiers in aerobic granules." Appl. Microbiol. Biotechnol. **85**(3): 753 - 762.

Agrawal, S., S. M. Karst, E. M. Gilbert, H. Horn, P. H. Nielsen and S. Lackner (2017). "The role of inoculum and reactor configuration for microbial community composition and dynamics in mainstream partial nitritation anammox reactors." MicrobiologyOpen **6**(4): e00456.

Ahmed, T. and M. J. Semmens (1992). "Use of sealed end hollow fibers for bubbleless membrane aeration: experimental studies." Journal of Membrane Science **69**(1-2): 1-10.

APHA AWWA, W. (1998). "Standard methods for the examination of water and wastewater 20th edition." American Public Health Association, American Water Work Association, Water Environment Federation, Washington, DC.

Aqeel, H., D. G. Weissbrodt, M. Cerruti, G. M. Wolfaardt, B.-M. Wilén and S. N. Liss (2019). "Drivers of bioaggregation from flocs to biofilms and granular sludge." Environmental Science: Water Research & Technology **5**(12): 2072-2089.

Awolusi, O., S. Kumari and F. Bux (2015). "Ecophysiology of nitrifying communities in membrane bioreactors." International Journal of Environmental Science and Technology **12**: 747-762.

Aybar, M., G. Pizarro, J. Boltz, L. Downing and R. Nerenberg (2014). "Energy-efficient wastewater treatment via the air-based, hybrid membrane biofilm reactor (hybrid MfBR)." Water science and technology **69**(8): 1735-1741.

Aybar, M., G. Pizarro, J. Boltz and R. Nerenberg (2015). Energy efficient wastewater treatment via the membrane-aerated biofilm reactor (MABR): Effect of membrane fluxes on performance. WEFTEC 2015, Water Environment Federation.

Bassin, J., R. Kleerebezem, A. Rosado, M. M. van Loosdrecht and M. Dezotti (2012). "Effect of different operational conditions on biofilm development, nitrification, and nitrifying microbial population in moving-bed biofilm reactors." Environmental Science & Technology **46**(3): 1546-1555.

Begmatov, S., A. G. Dorofeev, V. V. Kadnikov, A. V. Beletsky, N. V. Pimenov, N. V. Ravin and A. V. Mardanov (2022). "The structure of microbial communities of activated sludge of large-scale wastewater treatment plants in the city of Moscow." Scientific reports **12**(1): 3458.

Bian, X., Y. Wu, J. Li, M. Yin, D. Li, H. Pei, S. Chang and W. Guo (2022). "Effect of dissolved oxygen on high C/N wastewater treatment in moving bed biofilm reactors based on heterotrophic

nitrification and aerobic denitrification: Nitrogen removal performance and potential mechanisms." Bioresource Technology **365**: 128147.

Blackburne, R., Z. G. Yuan and J. Keller (2008). "Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor." Biodegradation. **19**.

Boltz, J. and G. Daigger (2010). "Uncertainty in bulk-liquid hydrodynamics and biofilm dynamics creates uncertainties in biofilm reactor design." Water Science and Technology **61**(2): 307-316.

Boltz, J. P., B. F. Smets, B. E. Rittmann, M. C. Van Loosdrecht, E. Morgenroth and G. T. Daigger (2017). "From biofilm ecology to reactors: a focused review." Water Science and Technology **75**(8): 1753-1760.

Bunse, P., L. Orschler, S. Agrawal and S. Lackner (2020). "Membrane aerated biofilm reactors for mainstream partial nitritation/anammox: Experiences using real municipal wastewater." Water Research X **9**: 100066.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson and S. P. Holmes (2016). "DADA2: High-resolution sample inference from Illumina amplicon data." Nature methods **13**(7): 581-583.

Canto-Encalada, G., D. Tec-Campos, J. D. Tibocha-Bonilla, K. Zengler, A. Zepeda and C. Zuñiga (2022). "Flux balance analysis of the ammonia-oxidizing bacterium Nitrosomonas europaea ATCC19718 unravels specific metabolic activities while degrading toxic compounds." PLoS Computational Biology **18**(2): e1009828.

Cao, C., L. Zhao, D. Xu and Q. Geng (2009). "Membrane-aerated biofilm reactor behaviors for the treatment of high-strength ammonium industrial wastewater." Chemical Engineering & Technology: Industrial Chemistry-Plant Equipment-Process Engineering-Biotechnology **32**(4): 613-621.

Carlson, A. L., H. He, C. Yang and G. T. Daigger (2021). "Comparison of hybrid membrane aerated biofilm reactor (MABR)/suspended growth and conventional biological nutrient removal processes." Water Science and Technology **83**(6): 1418-1428.

Carniello, V., B. W. Peterson, H. C. van der Mei and H. J. Busscher (2018). "Physico-chemistry from initial bacterial adhesion to surface-programmed biofilm growth." Advances in colloid and interface science **261**: 1-14.

Casey, E., B. Glennon and G. Hamer (1999). "Oxygen mass transfer characteristics in a membrane-aerated biofilm reactor." Biotechnology and bioengineering **62**(2): 183-192.

Casey, E., B. Glennon and G. Hamer (2000). "Biofilm development in a membrane-aerated biofilm reactor: Effect of flow velocity on performance." Biotechnology and Bioengineering **67**(4): 476-486.

Castagna, L., A. Zanella, V. Scaravilli, F. Magni, S. A. E. A. E. S. Deab, M. Introna, F. Mojoli, G. Grasselli, A. Pesenti and N. Patroniti (2015). "Effects on membrane lung gas exchange of an intermittent high gas flow recruitment maneuver: preliminary data in veno-venous ECMO patients." Journal of Artificial Organs **18**: 213-219.

Castrillo, M., R. Díez-Montero, A. L. Esteban-García and I. Tejero (2019). "Mass transfer enhancement and improved nitrification in MABR through specific membrane configuration." Water research **152**: 1-11.

Chai, H., Y. Xiang, R. Chen, Z. Shao, L. Gu, L. Li and Q. He (2019). "Enhanced simultaneous nitrification and denitrification in treating low carbon-to-nitrogen ratio wastewater: Treatment performance and nitrogen removal pathway." Bioresource technology **280**: 51-58.

Chen, R., S. Cao, L. Zhang and Y. Zhou (2022). "NOB suppression strategies in a mainstream membrane aerated biofilm reactor under exceptionally low lumen pressure." Chemosphere **290**: 133386.

Chen, R. and Y. Zhou (2021). "Measure microbial activity driven oxygen transfer in membrane aerated biofilm reactor from supply side." Environmental Research **195**: 110845.

Chen, Y., S. Lan, L. Wang, S. Dong, H. Zhou, Z. Tan and X. Li (2017). "A review: driving factors and regulation strategies of microbial community structure and dynamics in wastewater treatment systems." Chemosphere **174**: 173-182.

Choi, M., K. Cho, D. Jeong, Y.-C. Chung, J. Park, S. Lee and H. Bae (2018). "Effects of the ammonium loading rate on nitrite-oxidizing activity during nitrification at a high dose of inorganic carbon." Journal of Environmental Science and Health, Part A **53**(8): 708-717.

Cole, A. C., M. J. Semmens and T. M. LaPara (2004). "Stratification of activity and bacterial community structure in biofilms grown on membranes transferring oxygen." Applied and Environmental Microbiology **70**(4): 1982-1989.

Cole, A. C., J. W. Shanahan, M. J. Semmens and T. M. LaPara (2002). "Preliminary studies on the microbial community structure of membrane-aerated biofilms treating municipal wastewater." Desalination **146**(1-3): 421-426.

Corsino, S. F. and M. Torregrossa (2022). "Achieving complete nitrification below the washout SRT with hybrid membrane aerated biofilm reactor (MABR) treating municipal wastewater." Journal of Environmental Chemical Engineering **10**(1): 106983.

Cote, P., J.-L. Bersillon, A. Huyard and G. Faup (1988). "Bubble-free aeration using membranes: process analysis." Journal (Water Pollution Control Federation): 1986-1992.

Côté, P., J. Peeters, N. Adams, Y. Hong, Z. Long and J. Ireland (2015). "A new membrane-aerated biofilm reactor for low energy wastewater treatment: Pilot results." Proceedings of the Water Environment Federation **13**: 4226-4239.

Côté, P., J. Peeters, N. Adams, Y. Hong, Z. Long and J. Ireland (2015). A new membrane-aerated biofilm reactor for low energy wastewater treatment: pilot results. WEFTEC 2015, Water Environment Federation.

Cydzik-Kwiatkowska, A. and M. Zielińska (2016). "Bacterial communities in full-scale wastewater treatment systems." World Journal of Microbiology and Biotechnology **32**: 1-8.

Czieborowski, M., A. Hübenthal, A. Poehlein, I. Vogt and B. Philipp (2020). "Genetic and physiological analysis of biofilm formation on different plastic surfaces by Sphingomonas sp. strain S2M10 reveals an essential function of sphingan biosynthesis." Microbiology **166**(10): 918-935.

Da Silva, T. S., T. Matsumoto, M. L. Dos Anjos and L. L. Albertin (2018). "Organic matter removal in a membrane-aerated biofilm reactor." Journal of Environmental Engineering **144**(8): 04018057.

De-la-Pinta, I., M. Cobos, J. Ibarretxe, E. Montoya, E. Eraso, T. Guraya and G. Quindós (2019). "Effect of biomaterials hydrophobicity and roughness on biofilm development." Journal of Materials Science: Materials in Medicine **30**: 1-11.

De Meis, D. (2017). Gas transport through porous membranes, ENEA.

Denkhaus, E., S. Meisen, U. Telgheder and J. Wingender (2007). "Chemical and physical methods for characterisation of biofilms." Microchimica Acta **158**: 1-27.

Dodds, W. K. and E. B. Welch (2000). "Establishing nutrient criteria in streams." Journal of the North American Benthological Society **19**(1): 186-196.

Dong, W.-y., H.-j. Wang, W.-g. Li, W.-c. Ying, G.-h. Gan and Y. Yang (2009). "Effect of DO on simultaneous removal of carbon and nitrogen by a membrane aeration/filtration combined bioreactor." Journal of Membrane Science **344**(1-2): 219-224.

Downing, L. S. and R. Nerenberg (2008). "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.

Elsayed, A., M. Hurdle and Y. Kim (2021). "Comprehensive model applications for better understanding of pilot-scale membrane-aerated biofilm reactor performance." Journal of Water Process Engineering **40**: 101894.

Fitzgerald, C. M., P. Camejo, J. Z. Oshlag and D. R. Noguera (2015). "Ammonia-oxidizing microbial communities in reactors with efficient nitrification at low-dissolved oxygen." Water research **70**: 38-51.

Frølund, B., R. Palmgren, K. Keiding and P. H. Nielsen (1996). "Extraction of extracellular polymers from activated sludge using a cation exchange resin." Water research **30**(8): 1749-1758.

Ge, S., S. Wang, X. Yang, S. Qiu, B. Li and Y. Peng (2015). "Detection of nitrifiers and evaluation of partial nitrification for wastewater treatment: A review." Chemosphere **140**: 85-98.

Gilbert, E. M., S. Agrawal, F. Brunner, T. Schwartz, H. Horn and S. Lackner (2014). "Response of different Nitrospira species to anoxic periods depends on operational DO." Environmental science & technology **48**(5): 2934-2941.

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

Godzieba, M., M. Zubrowska-Sudol, J. Walczak and S. Ciesielski (2022). "Development of microbial communities in biofilm and activated sludge in a hybrid reactor." Scientific Reports **12**(1): 12558.

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

Grady Jr, C. L., G. T. Daigger, N. G. Love and C. D. Filipe (2011). Biological wastewater treatment, CRC press.

Guglielmi, G., D. Coutts, D. Houweling and J. Peeters (2020). "FULL-SCALE APPLICATION OF MABR TECHNOLOGY FOR UPGRADING AND RETROFITTING AN EXISTING WWTP: PERFORMANCES AND PROCESS MODELLING." Environmental Engineering & Management Journal (EEMJ) **19**(10).

He, H., B. M. Wagner, A. L. Carlson, C. Yang and G. T. Daigger (2021). "Recent progress using membrane aerated biofilm reactors for wastewater treatment." Water Science and Technology **84**(9): 2131-2157.

Heffernan, B., A. Shrivastava, D. Toniolo, M. Semmens and E. Syron (2017). Operation of a large scale membrane aerated biofilm reactor for the treatment of municipal wastewater. WEFTEC 2017, Water Environment Federation.

Heffernan, B., A. Shrivastava, D. Toniolo, M. Semmens and E. Syron (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.

Hibiya, K., A. Terada, S. Tsuneda and A. Hirata (2003). "Simultaneous nitrification and denitrification by controlling vertical and horizontal microenvironment in a membrane-aerated biofilm reactor." Journal of biotechnology **100**(1): 23-32.

Hoa, P., L. Nair and C. Visvanathan (2003). "The effect of nutrients on extracellular polymeric substance production and its influence on sludge properties." Water Sa **29**(4): 437-442.

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

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

Houweling, D. and G. T. Daigger (2019). Intensifying Activated Sludge Using Media-Supported Biofilms, CRC Press.

Houweling, D., J. Peeters, P. Cote, Z. Long and N. Adams (2017). "Proving membrane aerated biofilm reactor (MABR) performance and reliability: Results from four pilots and a full-scale plant." Proceedings of the Water Environment Federation **2017**(16): 272-284.

Houweling, D., J. Peeters, P. Cote, Z. Long and N. Adams (2017). Proving membrane aerated biofilm reactor (MABR) performance and reliability: Results from four pilots and a full-scale plant. WEFTEC 2017, Water Environment Federation.

Hu, S., F. Yang, S. Liu and L. Yu (2009). "The development of a novel hybrid aerating membrane-anaerobic baffled reactor for the simultaneous nitrogen and organic carbon removal from wastewater." Water Research **43**(2): 381-388.

Hu, X. b., K. Xu, Z. Wang, L. l. Ding and H. q. Ren (2013). "Characteristics of biofilm attaching to carriers in moving bed biofilm reactor used to treat vitamin C wastewater." Scanning **35**(5): 283-291.

Hu, Z., D. Houweling and P. Dold (2012). "Biological nutrient removal in municipal wastewater treatment: new directions in sustainability." Journal of Environmental Engineering **138**(3): 307-317.

Iorhemen, O. T., S. Ukaigwe, H. Dang and Y. Liu (2022). "Phosphorus Removal from Aerobic Granular Sludge: Proliferation of Polyphosphate-Accumulating Organisms (PAOs) under Different Feeding Strategies." Processes **10**(7): 1399.

Janczewski, L. and A. Trusek-Holownia (2016). "Biofilm-based membrane reactors–selected aspects of the application and microbial layer control." Desalination and Water Treatment **57**(48-49): 22909-22916.

Kelly, J. J., M. G. London, A. R. McCormick, M. Rojas, J. W. Scott and T. J. Hoellein (2021). "Wastewater treatment alters microbial colonization of microplastics." PloS one **16**(1): e0244443.

Kinh, C. T., S. Riya, M. Hosomi and A. Terada (2017). "Identification of hotspots for NO and N2O production and consumption in counter-and co-diffusion biofilms for simultaneous nitrification and denitrification." Bioresource technology **245**: 318-324.

Kitzinger, K., H. Koch, S. Lücker, C. J. Sedlacek, C. Herbold, J. Schwarz, A. Daebeler, A. J. Mueller, M. Lukumbuzya and S. Romano (2018). "Characterization of the first "Candidatus Nitrotoga" isolate reveals metabolic versatility and separate evolution of widespread nitrite-oxidizing bacteria." MBio **9**(4).

Koch, H., S. Lücker, M. Albertsen, K. Kitzinger, C. Herbold, E. Spieck, P. H. Nielsen, M. Wagner and H. Daims (2015). "Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus Nitrospira." Proceedings of the national academy of sciences **112**(36): 11371-11376.

Koskinen, R., T. Ali-Vehmas, P. Kämpfer, M. Laurikkala, I. Tsitko, E. Kostyal, F. Atroshi and M. Salkinoja-Salonen (2000). "Characterization of Sphingomonas isolates from Finnish and Swedish drinking water distribution systems." Journal of Applied Microbiology **89**(4): 687-696.

Kostera, J., M. D. Youngblut, J. M. Slosarczyk and A. A. Pacheco (2008). "Kinetic and product distribution analysis of NO· reductase activity in Nitrosomonas europaea hydroxylamine oxidoreductase." JBIC Journal of Biological Inorganic Chemistry **13**: 1073-1083.

Krause, S., B. Zimmermann, U. Meyer-Blumenroth, W. Lamparter, B. Siembida and P. Cornel (2010). "Enhanced membrane bioreactor process without chemical cleaning." Water Science and Technology **61**(10): 2575-2580.

Krishna, K. B., A. Sathasivan and M. P. Ginige (2013). "Microbial community changes with decaying chloramine residuals in a lab-scale system." Water research **47**(13): 4666-4679.

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

Kurisu, F., F. Kurisu, Y. Sakamoto and O. Yagi (2007). "Effects of ammonium and nitrite on communities and populations of ammonia-oxidizing bacteria in laboratory-scale continuous-flow reactors." FEMS microbiology ecology **60**(3): 501-512.

Lackner, S., A. Terada, H. Horn, M. Henze and B. F. Smets (2010). "Nitritation performance in membrane-aerated biofilm reactors differs from conventional biofilm systems." Water Research **44**(20): 6073-6084.

Lai, C.-Y., L. Zhong, Y. Zhang, J.-X. Chen, L.-L. Wen, L.-D. Shi, Y.-P. Sun, F. Ma, B. E. Rittmann and C. Zhou (2016). "Bioreduction of chromate in a methane-based membrane biofilm reactor." Environmental science & technology **50**(11): 5832-5839.

LaPara, T. M., A. C. Cole, J. W. Shanahan and M. J. Semmens (2006). "The effects of organic carbon, ammoniacal-nitrogen, and oxygen partial pressure on the stratification of membrane-aerated biofilms." Journal of Industrial Microbiology and Biotechnology **33**(4): 315-323.

Latocheski, E. C., M. C. V. da Rocha and M. C. B. Braga (2022). "Nitrospira in wastewater treatment: applications, opportunities and research gaps." Reviews in Environmental Science and Bio/Technology **21**(4): 905-930.

Lawson, C. E. and S. Lücker (2018). "Complete ammonia oxidation: an important control on nitrification in engineered ecosystems?" Current opinion in biotechnology **50**: 158-165.

Lema, J. M. and S. S. Martinez (2017). Innovative wastewater treatment & resource recovery technologies: impacts on energy, economy and environment, IWA publishing.

Li, J., L.-P. Zhu, Y.-Y. Xu and B.-K. Zhu (2010). "Oxygen transfer characteristics of hydrophilic treated polypropylene hollow fiber membranes for bubbleless aeration." Journal of Membrane Science **362**(1-2): 47-57.

Li, M., C. Du, J. Liu, X. Quan, M. Lan and B. Li (2018). "Mathematical modeling on the nitrogen removal inside the membrane-aerated biofilm dominated by ammonia-oxidizing archaea (AOA): effects of temperature, aeration pressure and COD/N ratio." Chemical Engineering Journal **338**: 680-687.

Li, M., Y. Li, N. Wang, T. Li, H. Guo, Z. Wu, P. Zhang, B. Wang and B. Li (2022). "Achieving efficient nitrogen removal in a single-stage partial nitrification-anammox-partial denitrification (PN/A/PD) membrane aerated biofilm reactor (MABR)." Journal of Water Process Engineering **49**: 103100.

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

Li, X., D. Bao, Y. Zhang, W. Xu, C. Zhang, H. Yang, Q. Ru, Y.-f. Wang, H. Ma and E. Zhu (2023). "Development and application of membrane aerated biofilm reactor (MABR)—A review." Water **15**(3): 436.

Li, Y. and K. Zhang (2018). "Pilot scale treatment of polluted surface waters using membrane-aerated biofilm reactor (MABR)." Biotechnology & Biotechnological Equipment **32**(2): 376-386.

Liang, Z., W. Li, S. Yang and P. Du (2010). "Extraction and structural characteristics of extracellular polymeric substances (EPS), pellets in autotrophic nitrifying biofilm and activated sludge." Chemosphere **81**(5): 626-632.

Liao, B. and S. Liss (2007). "A comparative study between thermophilic and mesophilic membrane aerated biofilm reactors." Journal of Environmental Engineering and Science **6**(2): 247-252.

Limpiyakorn, T., Y. Shinohara, F. Kurisu and O. Yagi (2005). "Communities of ammonia-oxidizing bacteria in activated sludge of various sewage treatment plants in Tokyo." FEMS microbiology ecology **54**(2): 205-217.

Lin, J., P. Zhang, G. Li, J. Yin, J. Li and X. Zhao (2016). "Effect of COD/N ratio on nitrogen removal in a membrane-aerated biofilm reactor." International Biodeterioration & Biodegradation **113**: 74-79.

Lin, J., P. Zhang, J. Yin, X. Zhao and J. Li (2015). "Nitrogen removal performances of a polyvinylidene fluoride membrane-aerated biofilm reactor." International Biodeterioration & Biodegradation **102**: 49-55.

Liu, G. and J. Wang (2013). "Long-term low DO enriches and shifts nitrifier community in activated sludge." Environmental science & technology **47**(10): 5109-5117.

Liu, H., S. Tan, Z. Sheng, Y. Liu and T. Yu (2014). "Bacterial community structure and activity of sulfate reducing bacteria in a membrane aerated biofilm analyzed by microsensor and molecular techniques." Biotechnology and Bioengineering **111**(11): 2155-2162.

Liu, H., F. Yang, T. Wang, Q. Liu and S. Hu (2007). "Carbon membrane-aerated biofilm reactor for synthetic wastewater treatment." Bioprocess and biosystems engineering **30**(4): 217-224.

Liu, W., W. Chen, D. Yang and Y. Shen (2019). "Functional and compositional characteristics of nitrifiers reveal the failure of achieving mainstream nitritation under limited oxygen or ammonia conditions." Bioresource technology **275**: 272-279.

Liu, W., Q. Yang, B. Ma, J. Li, L. Ma, S. Wang and Y. Peng (2017). "Rapid achievement of nitritation using aerobic starvation." Environmental Science & Technology **51**(7): 4001-4008.

Liu, X., M. Kim, G. Nakhla, M. Andalib and Y. Fang (2020). "Partial nitrification-reactor configurations, and operational conditions: Performance analysis." Journal of Environmental Chemical Engineering **8**(4): 103984.

Liu, Y., H. H. Ngo, W. Guo, L. Peng, D. Wang and B. Ni (2019). "The roles of free ammonia (FA) in biological wastewater treatment processes: A review." Environment international **123**: 10-19.

Long, Z., A. K. Oskouie, T. E. Kunetz, J. Peeters, N. Adams and D. Houweling (2020). "Simulation of long-term performance of an innovative membrane-aerated biofilm reactor." Journal of Environmental Engineering **146**(6): 04020041.

Lu, D., H. Bai, F. Kong, S. N. Liss and B. Liao (2021). "Recent advances in membrane aerated biofilm reactors." Critical Reviews in Environmental Science and Technology **51**(7): 649-703.

Lu, J.-J., Q. Shen, X.-Y. Li, F.-Y. Sun, J.-B. Yi, W.-Y. Dong, W.-J. Yan, H. Du and J.-L. Mu (2022). "Surface-manipulated membranes to accelerate biofilm formation and to resist bacterial detachment in MBfR for aerobic methane oxidation coupled to denitrification." Chemical Engineering Journal **430**: 132629.

Luo, Y.-H., R. Chen, L.-L. Wen, F. Meng, Y. Zhang, C.-Y. Lai, B. E. Rittmann, H.-P. Zhao and P. Zheng (2015). "Complete perchlorate reduction using methane as the sole electron donor and carbon source." Environmental science & technology **49**(4): 2341-2349.

Ma, Y., C. Domingo-Felez, B. G. Plósz and B. F. Smets (2017). "Intermittent aeration suppresses nitrite-oxidizing bacteria in membrane-aerated biofilms: a model-based explanation." Environmental science & technology **51**(11): 6146-6155.

Ma, Y., A. Piscedda, A. D. L. C. Veras, C. Domingo-Félez and B. F. Smets (2022). "Intermittent aeration to regulate microbial activities in membrane-aerated biofilm reactors: Energy-efficient nitrogen removal and low nitrous oxide emission." Chemical Engineering Journal **433**: 133630.

Maddela, N. R., Z. Gan, Y. Meng, F. Fan and F. Meng (2022). "Occurrence and roles of comammox bacteria in water and wastewater treatment systems: a critical review." Engineering **17**: 196-206.

Martin, K. J., J. P. Boltz and R. Nerenberg (2012). The Membrane Biofilm Reactor (MBfR) for Wastewater Treatment: Applications, Design Considerations, and Technology Outlook. Proceedings of 85th Annual Water Environment Federation Technical Exposition & Conference.

Martin, K. J. and R. Nerenberg (2012). "The membrane biofilm reactor (MBfR) for water and wastewater treatment: principles, applications, and recent developments." Bioresource technology **122**: 83-94.

McCarty, P. L., J. Bae and J. Kim (2011). Domestic wastewater treatment as a net energy producer–can this be achieved?, ACS Publications.

McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight and P. Hugenholtz (2012). "An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea." The ISME journal **6**(3): 610-618.

Mehrabi, S., D. Houweling and M. Dagnew (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.

Melo, L. F. (2003). "Biofilm formation and its role in fixed film processes." book: Water Wastewater Microbiology: 337-349.

Metcalf, Eddy, M. Abu-Orf, G. Bowden, F. L. Burton, W. Pfrang, H. D. Stensel, G. Tchobanoglous, R. Tsuchihashi and AECOM (2014). Wastewater engineering: treatment and resource recovery, McGraw Hill Education.

Miao, Y., L. Zhang, Y. Yang, Y. Peng, B. Li, S. Wang and Q. Zhang (2016). "Start-up of single-stage partial nitrification-anammox process treating low-strength swage and its restoration from nitrate accumulation." Bioresource Technology **218**: 771-779.

Mielczarek, A. T., H. T. T. Nguyen, J. L. Nielsen and P. H. Nielsen (2013). "Population dynamics of bacteria involved in enhanced biological phosphorus removal in Danish wastewater treatment plants." Water research **47**(4): 1529-1544.

Ming, T., R. De_richter, S. Shen and S. Caillol (2016). "Fighting global warming by greenhouse gas removal: destroying atmospheric nitrous oxide thanks to synergies between two breakthrough technologies." Environmental Science and Pollution Research **23**: 6119-6138.

Mohan, S. M. and S. Nagalakshmi (2020). "A review on aerobic self-forming dynamic membrane bioreactor: Formation, performance, fouling and cleaning." Journal of Water Process Engineering **37**: 101541.

Mota, C., J. Ridenoure, J. Cheng and F. L. de los Reyes III (2005). "High levels of nitrifying bacteria in intermittently aerated reactors treating high ammonia wastewater." FEMS Microbiology Ecology **54**(3): 391-400.

Nancharaiah, Y. V. and M. Sarvajith (2019). "Aerobic granular sludge process: a fast growing biological treatment for sustainable wastewater treatment." Current Opinion in Environmental Science & Health **12**: 57-65.

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

Nerenberg, R. (2005). Membrane biofilm reactors for water and wastewater treatment. Borchardt Conference: A Seminar on Advances in Water and Wastewater Treatment, Ann Arbor. MI.

Nerenberg, R. (2016). "The membrane-biofilm reactor (MBfR) as a counter-diffusional biofilm process." Current opinion in biotechnology **38**: 131-136.

Nisola, G. M., J. Orata-Flor, S. Oh, N. Yoo and W.-J. Chung (2013). "Partial nitrification in a membrane-aerated biofilm reactor with composite PEBA/PVDF hollow fibers." Desalination and Water Treatment **51**(25-27): 5275-5282.

Ohandja, D.-G. and D. C. Stuckey (2007). "Biodegradation of PCE in a hybrid membrane aerated biofilm reactor." Journal of Environmental Engineering **133**(1): 20-27.

Okabe, S., Y. Aoi, H. Satoh and Y. Suwa (2011). "Nitrification in wastewater treatment." Nitrification: 405-433.

Ontiveros-Valencia, A., C. Zhou, H.-P. Zhao, R. Krajmalnik-Brown, Y. Tang and B. Rittmann (2018). "Managing microbial communities in membrane biofilm reactors." Applied microbiology and biotechnology **102**: 9003-9014.

Pankhania, M., T. Stephenson and M. J. Semmens (1994). "Hollow fibre bioreactor for wastewater treatment using bubbleless membrane aeration." Water research **28**(10): 2233-2236.

Park, S., W. Bae and B. E. Rittmann (2010). "Operational boundaries for nitrite accumulation in nitrification based on minimum/maximum substrate concentrations that include effects of oxygen limitation, pH, and free ammonia and free nitrous acid inhibition." Environmental science & technology **44**(1): 335-342.

Park, S., J. Chung, B. E. Rittmann and W. Bae (2015). "Nitrite accumulation from simultaneous free-ammonia and free-nitrous-acid inhibition and oxygen limitation in a continuous-flow biofilm reactor." Biotechnology and Bioengineering **112**(1): 43-52.

Paśmionka, I. B., K. Bulski, P. Herbut, E. Boligłowa, F. M. C. Vieira, G. Bonassa, M. Bortoli and M. C. d. Prá (2021). "Toxic effect of ammonium nitrogen on the nitrification process and acclimatisation of nitrifying bacteria to high concentrations of NH4-N in wastewater." Energies **14**(17): 5329.

Pathak, S., S. Wang and E. Janka (2022). "Achieving Partial Nitritation in Anammox Start-Up Environment." Water **14**(2): 229.

Paul, E., J. C. Ochoa, Y. Pechaud, Y. Liu and A. Liné (2012). "Effect of shear stress and growth conditions on detachment and physical properties of biofilms." Water research **46**(17): 5499-5508.

Peeters, J., Z. Long, D. Houweling, P. Côté, G. T. Daigger and S. Snowling (2017). Nutrient removal intensification with MABR–developing a process model supported by piloting. Nutrient Removal and Recovery Symposium 2017, Water Environment Federation.

Pellicer-Nàcher, C. and B. F. Smets (2014). "Structure, composition, and strength of nitrifying membrane-aerated biofilms." water research **57**: 151-161.

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

Perez-Calleja, P., M. Aybar, C. Picioreanu, A. L. Esteban-Garcia, K. J. Martin and R. Nerenberg (2017). "Periodic venting of MABR lumen allows high removal rates and high gas-transfer efficiencies." Water research **121**: 349-360.

Pérez-Calleja, P., E. Clements and R. Nerenberg (2022). "Enhancing ammonium oxidation fluxes and nitritation efficiencies in MABRs: a modeling study." Environmental Science: Water Research & Technology **8**(2): 358-374.

Picioreanu, C., M. C. Van Loosdrecht and J. J. Heijnen (2001). "Two-dimensional model of biofilm detachment caused by internal stress from liquid flow." Biotechnology and bioengineering **72**(2): 205-218.

Pierce, J. and C. Suelter (1977). "An evaluation of the Coomassie brilliant blue G-250 dye-binding method for quantitative protein determination." Analytical Biochemistry **81**(2): 478-480.

Prosser, J. I., I. M. Head and L. Y. Stein (2014). The family nitrosomonadaceae. The prokaryotes: alphaproteobacteria and betaproteobacteria, Springer Berlin/Heidelberg**:** 901-918.

Qadir, M., P. Drechsel, B. Jiménez Cisneros, Y. Kim, A. Pramanik, P. Mehta and O. Olaniyan (2020). Global and regional potential of wastewater as a water, nutrient and energy source. Natural resources forum, Wiley Online Library.

Ravishankar, H., A. Nemeth, G. Massons, D. Puig, D. Zardoya, N. Carpi, P. N. Lens and B. Heffernan (2022). "Factors impacting simultaneous nitrification and denitrification in a membrane aerated biofilm reactor (MABR) system treating municipal wastewater." Journal of Environmental Chemical Engineering **10**(5): 108120.

Regmi, P., M. W. Miller, B. Holgate, R. Bunce, H. Park, K. Chandran, B. Wett, S. Murthy and C. B. Bott (2014). "Control of aeration, aerobic SRT and COD input for mainstream nitritation/denitritation." Water research **57**: 162-171.

Rittmann, B. E. and P. L. McCarty (1980). "Model of steady-state-biofilm kinetics." Biotechnology and bioengineering **22**(11): 2343-2357.

Rothemund, C., A. K. Camper and P. Wilderer (1994). "Biofilms growing on gas permeable membranes."

Rout, P. R., M. K. Shahid, R. R. Dash, P. Bhunia, D. Liu, S. Varjani, T. C. Zhang and R. Y. Surampalli (2021). "Nutrient removal from domestic wastewater: A comprehensive review on conventional and advanced technologies." Journal of Environmental Management **296**: 113246.

Salbitani, G. and S. Carfagna (2021). "Ammonium utilization in microalgae: A sustainable method for wastewater treatment." Sustainability **13**(2): 956.

Sanchez-Huerta, C., L. Fortunato, T. Leiknes and P.-Y. Hong (2022). "Influence of biofilm thickness on the removal of thirteen different organic micropollutants via a Membrane Aerated Biofilm Reactor (MABR)." Journal of Hazardous Materials **432**: 128698.

Scherson, Y. D., G. F. Wells, S.-G. Woo, J. Lee, J. Park, B. J. Cantwell and C. S. Criddle (2013). "Nitrogen removal with energy recovery through N 2 O decomposition." Energy & Environmental Science **6**(1): 241-248.

Schramm, A., D. De Beer, A. Gieseke and R. Amann (2000). "Microenvironments and distribution of nitrifying bacteria in a membrane-bound biofilm." Environmental microbiology **2**(6): 680-686.

Sehar, S. and I. Naz (2016). "Role of the biofilms in wastewater treatment." Microbial biofilms-importance and applications: 121-144.

Semmens, M. (2005). "Membrane technology: pilot studies of membrane-aerated bioreactors."

Semmens, M. and D. Hanus (1999). "Studies of a membrane aerated bioreactor for wastewater treatment." Membrane Technology **1999**(111): 9-13.

Semmens, M. and J. Shanahan (2005). "Membrane Technology: Pilot Studies of Membrane Aerated Bioreactors, Final Report." Water Environment Research Foundation, Virginia, USA.

Semmens, M. J., K. Dahm, J. Shanahan and A. Christianson (2003). "COD and nitrogen removal by biofilms growing on gas permeable membranes." Water Research **37**(18): 4343-4350.

Shanahan, J. and M. Semmens (2006). "Influence of a nitrifying biofilm on local oxygen fluxes across a micro-porous flat sheet membrane." Journal of Membrane Science **277**(1-2): 65-74.

Shanahan, J. W. (2007). Modeling and Experimental Analyses of Membrane-aerated Biofilms, University of Minnesota.

Shanahan, J. W. and M. J. Semmens (2015). "Alkalinity and pH effects on nitrification in a membrane aerated bioreactor: an experimental and model analysis." Water Research **74**: 10-22.

Shaowei, H., Y. Fenglin, S. Cui, J. Zhang and W. Tonghua (2008). "Simultaneous removal of COD and nitrogen using a novel carbon-membrane aerated biofilm reactor." Journal of Environmental Sciences **20**(2): 142-148.

Sharif Shourjeh, M., P. Kowal, X. Lu, L. Xie and J. Drewnowski (2021). "Development of strategies for AOB and NOB competition supported by mathematical modeling in terms of successful deammonification implementation for energy-efficient WWTPs." Processes **9**(3): 562.

Shi, Y., J. Huang, G. Zeng, Y. Gu, Y. Chen, Y. Hu, B. Tang, J. Zhou, Y. Yang and L. Shi (2017). "Exploiting extracellular polymeric substances (EPS) controlling strategies for performance enhancement of biological wastewater treatments: an overview." Chemosphere **180**: 396-411.

Shinoda, Y., Y. Sakai, H. Uenishi, Y. Uchihashi, A. Hiraishi, H. Yukawa, H. Yurimoto and N. Kato (2004). "Aerobic and anaerobic toluene degradation by a newly isolated denitrifying bacterium, Thauera sp. strain DNT-1." Applied and Environmental Microbiology **70**(3): 1385-1392.

Shoji, T., R. Itoh, T. Nittami, T. Kageyama, M. Noguchi and A. Yamasaki (2020). "Influence of the flow velocity on membrane-aerated biofilm reactors: application of a rotating disk for local flow control." Biochemical Engineering Journal **164**: 107771.

Silveira, I. T., K. Cadee and W. Bagg (2022). "Startup and initial operation of an MLE-MABR treating municipal wastewater." Water Science and Technology **85**(4): 1155-1166.

Sliekers, A. O., S. C. Haaijer, M. H. Stafsnes, J. G. Kuenen and M. S. Jetten (2005). "Competition and coexistence of aerobic ammonium-and nitrite-oxidizing bacteria at low oxygen concentrations." Applied microbiology and biotechnology **68**: 808-817.

Spasov, E., J. M. Tsuji, L. A. Hug, A. C. Doxey, L. A. Sauder, W. J. Parker and J. D. Neufeld (2020). "High functional diversity among Nitrospira populations that dominate rotating biological contactor microbial communities in a municipal wastewater treatment plant." The ISME Journal **14**(7): 1857-1872.

Spieck, E., S. Keuter, T. Wenzel, E. Bock and W. Ludwig (2014). "Characterization of a new marine nitrite oxidizing bacterium, Nitrospina watsonii sp. nov., a member of the newly proposed phylum "Nitrospinae"." Systematic and applied microbiology **37**(3): 170-176.

Stricker, A. E., H. Lossing, J. H. Gibson, Y. Hong and J. C. Urbanic (2011). "Pilot Scale Testing of A New Configuration of The Membrane Aerated Biofilm Reactor (MABR) to Treat High-Strength Industrial Sewage." Water Environment Research **83**(1): 3-14.

Suarez, C., M. Piculell, O. Modin, S. Langenheder, F. Persson and M. Hermansson (2019). "Thickness determines microbial community structure and function in nitrifying biofilms via deterministic assembly." Scientific reports **9**(1): 1-10.

Sun, L., Z. Wang, X. Wei, P. Li, H. Zhang, M. Li, B. Li and S. Wang (2015). "Enhanced biological nitrogen and phosphorus removal using sequencing batch membrane-aerated biofilm reactor." Chemical Engineering Science **135**: 559-565.

Sun, S.-P., C. P. i. Nàcher, B. Merkey, Q. Zhou, S.-Q. Xia, D.-H. Yang, J.-H. Sun and B. F. Smets (2010). "Effective biological nitrogen removal treatment processes for domestic wastewaters with low C/N ratios: a review." Environmental Engineering Science **27**(2): 111-126.

Sun, Z., M. Li, G. Wang, X. Yan, Y. Li, M. Lan, R. Liu and B. Li (2020). "Enhanced carbon and nitrogen removal in an integrated anaerobic/anoxic/aerobic-membrane aerated biofilm reactor system." RSC advances **10**(48): 28838-28847.

Sun, Z., Y. Li, M. Li, N. Wang, J. Liu, H. Guo and B. Li (2022). "Steel pickling rinse wastewater treatment by two-stage MABR system: Reactor performance, extracellular polymeric substances (EPS) and microbial community." Chemosphere **299**: 134402.

Sunner, N., Z. Long, D. Houweling, A. Monti and J. Peeters (2018). MABR as a low-energy compact solution for nutrient removal upgrades—results from a demonstration in the UK. WEFTEC 2018, Water Environment Federation.

Suzuki, Y., N. Hatano, S. Ito and H. Ikeda (2000). "Performance of nitrogen removal and biofilm structure of porous gas permeable membrane reactor." Water science and technology **41**(4-5): 211-217.

Syron, E. and E. Casey (2008). "Membrane-aerated biofilms for high rate biotreatment: performance appraisal, engineering principles, scale-up, and development requirements." Environmental science & technology **42**(6): 1833-1844.

Syron, E. and B. Heffernan (2017). OxyMem, The Flexiable MABR. Nutrient Removal and Recovery Symposium 2017, Water Environment Federation.

Syron, E., M. J. Semmens and E. Casey (2015). "Performance analysis of a pilot-scale membrane aerated biofilm reactor for the treatment of landfill leachate." Chemical Engineering Journal **273**: 120-129.

Taşkan, B., H. Hasar and C. H. Lee (2020). "Effective biofilm control in a membrane biofilm reactor using a quenching bacterium (Rhodococcus sp. BH4)." Biotechnology and Bioengineering **117**(4): 1012-1023.

Tay, J.-H., Q.-S. Liu and Y. Liu (2002). "Aerobic granulation in sequential sludge blanket reactor." Water Science and Technology **46**(4-5): 13-18.

Terada, A., K. Hibiya, J. Nagai, S. Tsuneda and A. Hirata (2003). "Nitrogen removal characteristics and biofilm analysis of a membrane-aerated biofilm reactor applicable to high-strength nitrogenous wastewater treatment." Journal of bioscience and bioengineering **95**(2): 170-178.

Terada, A., S. Lackner, K. Kristensen and B. F. Smets (2010). "Inoculum effects on community composition and nitritation performance of autotrophic nitrifying biofilm reactors with counter-diffusion geometry." Environmental Microbiology **12**(10): 2858-2872.

Terada, A., S. Sugawara, T. Yamamoto, S. Zhou, K. Koba and M. Hosomi (2013). "Physiological characteristics of predominant ammonia-oxidizing bacteria enriched from bioreactors with different influent supply regimes." Biochemical engineering journal **79**: 153-161.

Terada, A., T. Yamamoto, R. Igarashi, S. Tsuneda and A. Hirata (2006). "Feasibility of a membrane-aerated biofilm reactor to achieve controllable nitrification." Biochemical engineering journal **28**(2): 123-130.

Tian, H.-L., J.-Y. Zhao, H.-Y. Zhang, C.-Q. Chi, B.-A. Li and X.-L. Wu (2015). "Bacterial community shift along with the changes in operational conditions in a membrane-aerated biofilm reactor." Applied microbiology and biotechnology **99**: 3279-3290.

Tian, H., M. Hui, P. Pan, J. Huang, L. Chen and J. Zhao (2021). "Performance and microbial ecology of biofilms adhering on aerated membrane with distinctive conditions for the treatment of domestic sewage." Environmental Technology **42**(3): 459-467.

Tian, H., H. Zhang, P. Li, L. Sun, F. Hou and B. Li (2015). "Treatment of pharmaceutical wastewater for reuse by coupled membrane-aerated biofilm reactor (MABR) system." RSC advances **5**(85): 69829-69838.

Timberlake, D. L., S. E. Strand and K. J. Williamson (1988). "Combined aerobic heterotrophic oxidation, nitrification and denitrification in a permeable-support biofilm." Water research **22**(12): 1513-1517.

Ukaigwe, S., Y. Zhou, M. Shaheen and Y. Liu (2021). "Municipal wastewater treatment using a membrane aerated biofilm reactor." Journal of Environmental Engineering and Science **17**(2): 99-107.

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

Uri-Carreño, N., P. H. Nielsen, K. V. Gernaey and X. Flores-Alsina (2021). "Long-term operation assessment of a full-scale membrane-aerated biofilm reactor under Nordic conditions." Science of the Total Environment **779**: 146366.

Verstraete, W. and S. E. Vlaeminck (2011). "ZeroWasteWater: short-cycling of wastewater resources for sustainable cities of the future." International Journal of Sustainable Development & World Ecology **18**(3): 253-264.

Wagner, B., G. Daigger and N. Love (2022). "Assessing membrane aerated biofilm reactor configurations in mainstream anammox applications." Water Science and Technology **85**(3): 943-960.

Wang, B., S. Liu, X. Yao, Y. Liu and G. Qi (2022). "Alternating aeration strategy to reduce aeration energy demand for aerobic granular sludge and analysis of microbial community dynamics." Environmental Science: Water Research & Technology **8**(5): 1111-1125.

Wang, H., G. Xu, Z. Qiu, Y. Zhou and Y. Liu (2019). "NOB suppression in pilot-scale mainstream nitritation-denitritation system coupled with MBR for municipal wastewater treatment." Chemosphere **216**: 633-639.

Wang, J., J. Liang, L. Sun, J. Shen and M. Wang (2021). "Achieving reliable partial nitrification and anammox process using polyvinyl alcohol gel beads to treat low-strength ammonia wastewater." Bioresource technology **324**: 124669.

Wang, R., A. Terada, S. Lackner, B. F. Smets, M. Henze, S. Xia and J. Zhao (2009). "Nitritation performance and biofilm development of co-and counter-diffusion biofilm reactors: modeling and experimental comparison." Water research **43**(10): 2699-2709.

Wegen, S., B. Nowka and E. Spieck (2019). "Low temperature and neutral pH define “Candidatus Nitrotoga sp.” as a competitive nitrite oxidizer in coculture with Nitrospira defluvii." Applied and environmental microbiology **85**(9).

Werner, J. J., O. Koren, P. Hugenholtz, T. Z. DeSantis, W. A. Walters, J. G. Caporaso, L. T. Angenent, R. Knight and R. E. Ley (2012). "Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys." The ISME journal **6**(1): 94-103.

Wett, B., A. Omari, S. Podmirseg, M. Han, O. Akintayo, M. Gómez Brandón, S. Murthy, C. Bott, M. Hell and I. Takács (2013). "Going for mainstream deammonification from bench to full scale for maximized resource efficiency." Water science and technology **68**(2): 283-289.

Wu, G. and Q. Yin (2020). "Microbial niche nexus sustaining biological wastewater treatment." npj Clean Water **3**(1): 33.

Wu, J. and V. Chen (2000). "Shell-side mass transfer performance of randomly packed hollow fiber modules." Journal of Membrane Science **172**(1-2): 59-74.

Wu, Y., Z. Wu, H. Chu, J. Li, H. H. Ngo, W. Guo, N. Zhang and H. Zhang (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.

Xia, S., F. Zhong, Y. Zhang, H. Li and X. Yang (2010). "Bio-reduction of nitrate from groundwater using a hydrogen-based membrane biofilm reactor." Journal of Environmental Sciences **22**(2): 257-262.

Xiao, P., J. Zhou, X. Luo, B. Kang, L. Guo, G. Yuan, L. Zhang and T. Zhao (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.

Yamagiwa, K., A. Ohkawa and O. Hirasa (1994). "Simultaneous organic carbon removal and nitrification by biofilm formed on oxygen enrichment membrane." Journal of chemical engineering of Japan **27**(5): 638-643.

Yang, Q., Y. Peng, X. Liu, W. Zeng, T. Mino and H. Satoh (2007). "Nitrogen removal via nitrite from municipal wastewater at low temperatures using real-time control to optimize nitrifying communities." Environmental science & technology **41**(23): 8159-8164.

Yang, S., S. Xu, A. Mohammed, B. Guo, S. Vincent, N. J. Ashbolt and Y. Liu (2019). "Anammox reactor optimization for the treatment of ammonium rich digestate lagoon supernatant-Step feeding mitigates nitrite inhibition." International biodeterioration & biodegradation **143**: 104733.

Yeh, S.-J. and C. R. Jenkins (1978). "Pure oxygen fixed film reactor." Journal of the Environmental Engineering Division **104**(4): 611-623.

Yim, M.-S., Y. C. Yau, A. Matlow, J.-S. So, J. Zou, C. A. Flemming, H. Schraft and K. T. Leung (2010). "A novel selective growth medium-PCR assay to isolate and detect Sphingomonas in environmental samples." Journal of microbiological methods **82**(1): 19-27.

Yin, Q., Y. Sun, B. Li, Z. Feng and G. Wu (2022). "The r/K selection theory and its application in biological wastewater treatment processes." Science of the Total Environment: 153836.

Zhang, B., Q. Yu, G. Yan, H. Zhu, X. Y. Xu and L. Zhu (2018). "Seasonal bacterial community succession in four typical wastewater treatment plants: correlations between core microbes and process performance." Scientific reports **8**(1): 4566.

Zhang, H., Y. Jia, S. K. Khanal, H. Lu, H. Fang and Q. Zhao (2018). "Understanding the role of extracellular polymeric substances on ciprofloxacin adsorption in aerobic sludge, anaerobic sludge, and sulfate-reducing bacteria sludge systems." Environmental science & technology **52**(11): 6476-6486.

Zhang, L.-Q., X. Jiang, H.-W. Rong, C.-H. Wei, M. Luo, W.-C. Ma and H.-Y. Ng (2022). "Exploring the carbon and nitrogen removal capacity of a membrane aerated biofilm reactor for low-strength municipal wastewater treatment." Environmental Science: Water Research & Technology **8**(2): 280-289.

Zhang, P., B. Feng, Y.-P. Chen, Y.-Z. Dai and J.-S. Guo (2019). "In situ characterizations for EPS-involved microprocesses in biological wastewater treatment systems." Critical Reviews in Environmental Science and Technology **49**(11): 917-946.

Zhang, P., J.-S. Guo, Y. Shen, P. Yan, Y.-P. Chen, H. Wang, J.-X. Yang, F. Fang and C. Li (2015). "Microbial communities, extracellular proteomics and polysaccharides: a comparative investigation on biofilm and suspended sludge." Bioresource Technology **190**: 21-28.

Zhang, X. and Y. Liu (2022). "Circular economy is game-changing municipal wastewater treatment technology towards energy and carbon neutrality." Chemical Engineering Journal **429**: 132114.

Zhao, H., Y. Guo, Q. Wang, Z. Zhang, C. Wu, M. Gao and F. Liu (2022). "The Summary of Nitritation Process in Mainstream Wastewater Treatment." Sustainability **14**(24): 16453.

Zheng, M., S. Li, G. Ni, J. Xia, S. Hu, Z. Yuan, Y. Liu and X. Huang (2020). "Critical factors facilitating Candidatus Nitrotoga to be prevalent nitrite-oxidizing bacteria in activated sludge." Environmental science & technology **54**(23): 15414-15423.

Zheng, X., S. Zhang, J. Zhang, D. Huang and Z. Zheng (2018). "Advanced nitrogen removal from municipal wastewater treatment plant secondary effluent using a deep bed denitrification filter." Water Science and Technology **77**(11): 2723-2732.

Zhou, C., A. Ontiveros-Valencia, R. Nerenberg, Y. Tang, D. Friese, R. Krajmalnik-Brown and B. E. Rittmann (2019). "Hydrogenotrophic microbial reduction of oxyanions with the membrane biofilm reactor." Frontiers in microbiology **9**: 3268.

Zhou, S., C. Wei, C. Liao and H. Wu (2010). "Comprehensive study on dynamics of microbial community in Anaerobic-Oxic-Oxic process using PCR-DGGE, gas chromatography analysis, and dehydrogenase activity assays." World Journal of Microbiology and Biotechnology **26**: 273-279.

Zhou, Y., P. D. Kiely, R. Kibbee and B. Ormeci (2021). "Effect of polymeric support material on biofilm development, bacterial population, and wastewater treatment performance in anaerobic fixed-film systems." Chemosphere **264**: 128477.

Zhou, Y., R. Li, B. Guo, S. Xia, Y. Liu and B. E. Rittmann (2022). "The influent COD/N ratio controlled the linear alkylbenzene sulfonate biodegradation and extracellular polymeric substances accumulation in an oxygen-based membrane biofilm reactor." Journal of hazardous materials **422**: 126862.

Zhou, Y., R. Li, B. Guo, L. Zhang, X. Zou, S. Xia and Y. Liu (2020). "Greywater treatment using an oxygen-based membrane biofilm reactor: Formation of dynamic multifunctional biofilm for organics and nitrogen removal." Chemical Engineering Journal **386**: 123989.

Zhu, L., J. Zhou, M. Lv, H. Yu, H. Zhao and X. Xu (2015). "Specific component comparison of extracellular polymeric substances (EPS) in flocs and granular sludge using EEM and SDS-PAGE." Chemosphere **121**: 26-32.

Zhuang, H., Z. Wu, L. Xu, S.-Y. Leu and P.-H. Lee (2020). "Energy-efficient single-stage nitrite shunt denitrification with saline sewage through concise dissolved oxygen (DO) supply: process performance and microbial communities." Microorganisms **8**(6): 919.

Zou, X., Y. Zhou, M. Gao, S. Yang, A. Mohammed and Y. Liu (2022). "Effective N2O emission control during the nitritation/denitritation treatment of ammonia rich wastewater." Journal of Environmental Chemical Engineering **10**(2): 107234.

Chapter 7 – Conclusions and Recommendation for Future Work

7.1. Conclusions

This study investigated the operations of a conventional MABR for mainstream and high-strength wastewater treatments, with respect to process parameter optimization, biofilm thickness management, nitrogen removal pathways and microbial community dynamics. Two lab-scale MABRs were operated continuously for about 250 days and their performance assessed with respect to the research objectives. The main conclusions from the study are summarized below.

7.1.1. Optimization of operational parameter

- HRT has significant impact on both MABR performance and microbial community dynamics within the biofilm.
- MABR operation has often been performed at high HRT (low surface loading), although the system has the potentiality to handle higher surface loads (lower HRT).
- At optimized HRT of 3h, this MABR produced TCOD, NH_4^+-N and TN removal efficiencies of 98, 96 and 67% respectively with acceptable effluent discharge quality.
- At low HRT of 3h, MABR treating mainstream wastewater sustained sufficient active microbial populations to maintain high performance and stability over an extended period.

7.1.2. Biofilm thickness management

- MABR biofilm remains a critical component of the MABR technology. While effective biofilm thickness management remains a lingering challenge.
- Several biofilm thickness control strategies have been investigated for the MABR technology, which although resulted in efficient control of biofilm thickness but extended performance lag ensued inadvertently.

- A new biofilm thickness control strategy involving non-aggressive cleaning was developed in this study, which enabled the removal of peripheral biofilm, thereby checking biofilm overgrowth.
- The procedure prevented sloughing, consequently the loss of active biofilm microbes was minimized.
- The procedure also helped in the control of septicity by initiating membrane cleaning when bulk DO drop to set-point of 0.2mg/L.
- When applied in MABR operation, the procedure sustained biofilm with adequate thickness to produce mean removal efficiencies of 92, 99 and 84 % for COD, NH_4^+-N and TIN within a short HRT of 2.5 h and mean surface loading rates of 10 gCOD/m^2/d and 0.93 gN/m^2/d respectively.
- Microbial population at each stage of study indicated sufficient biodiversity and relative abundance for stable performance over extended operation periods.

7.1.3. Nitritation in MABR

- Whereas low DO condition can be applied to achieve nitritation in MABR for the removal of nitrogen from mainstream wastewater, however intermittent aeration is required for the sustenance of the process for extended operational time.
- Switching the process air supply mode from continuous to intermittent, can switch the MABR system from nitratating to nitritating.
- Using a combination of continuous and intermittent aeration modes with aerated and non-aerated cycles of 10 (5 on: 5 off), 20 (10 on: 10 off) and 25 (10 on: 15 off) minutes respectively. Nitrite accumulation rate (NAR), nitrate production rate (NPR) and

ammonium nitrogen removal efficiency (ANRE) of 35%, 12% and 99%; 76%, 3.4% and 98%; 94%, 1% and 98% respectively were obtained for Phases II - IV studied.

- Although this MABR could not successfully handle 25 minutes of aerated and non-aerated cycle but with 20 minutes, NPR was significantly reduced to 1%, more than 95% of the influent ammonium nitrogen was removed while NAR and TIN removal efficiency increased above 90 and 80% respectively for >100 days of MABR operation.
- Microbial activity analysis showed that low DO condition significantly reduced NOB activities without impacting AOB activities.

7.1.4. Combined inocula

- Mixed-inocula could contain microbial population of higher biodiversity while continuous recirculation of rigid AGS granules may produce shear with significant cumulative influence to regulate biofilm growth.
- Fluorescence microscopy revealed colonies with low number of viable cells in the biofilms, while SEM and TEM revealed non-dense and non-homogenously distributed biofilm on the membrane surfaces.
- Biofilm thickness of 0.357mm was obtained at the end of the study, which is lower than 0.5mm required for simultaneous nitrification-denitrification, therefore, both biofilm stratification and denitrification may have been inhibited.
- COD removal efficiencies were higher at all conditions studied, than $NH4^+$–N and TIN removal efficiencies, thus effect of the biofilm thickness was more consequential on $NH4^+$–N and TIN removal than on COD removal efficiency.

- Thickness control mechanisms applied may have inhibited biofilm growth instead of controlling overgrowth.
- The microbial community biodiversity showed minimal changes over the course of the study.
- More investigations are need to corroborate and validate the results obtained in this study.

7.2. Recommendations

This work was focused on improving MABR operations. The importance of biofilm control was identified and strategies for biofilm thickness management was developed both for mainstream and high-strength wastewaters. DO-based control strategy for stable nitritation in MABR was also developed. Based on study results and experiences, recommendations for future study are list below.

1. All the studies were done at lab-scale; as such, the results are susceptible to uncertainties associated with small reactors. It is therefore recommended that future studies be done with reactors representative of actual situations.
2. Membrane fibers were only removed for biofilm analysis at the end of the study due to the size of the reactor which made stage by stage analysis impracticable. Additionally, the different layers within the biofilm could not be isolated for specific analysis. It is therefore strongly suggested that reactors that allow for both stage by stage and in situ analysis without destabilizing the system be applied in future studies.
3. Although, the initial studies started with real wastewater primary effluent, but synthetic wastewater was used for most part of this study. Synthetic wastewater contains easily biodegradable pollutants, which may not be representative of real wastewater condition

where complex pollutants are encountered, therefore, studies may present over simplification of actual situations. It is therefore strongly recommended that real wastewater be used for future studies, so that all possible challenges are encountered during the study periods and tackled.

4. The study on combining CAS and AGS sludge in MABR process to check biofilm growth showed great potential for biofilm thickness control, therefore it is strongly recommended that further studies be focused in this critical area as the challenge of biofilm thickness management persists

Bibliography

Adav, S. S., D. J. Lee and J. Y. Lai (2010). "Microbial community of acetate utilizing denitrifiers in aerobic granules." Appl. Microbiol. Biotechnol. 85(3): 753 - 762.

Agrawal, S., S. M. Karst, E. M. Gilbert, H. Horn, P. H. Nielsen and S. Lackner (2017). "The role of inoculum and reactor configuration for microbial community composition and dynamics in mainstream partial nitritation anammox reactors." MicrobiologyOpen 6(4): e00456.

Ahmed, T. and M. J. Semmens (1992). "Use of sealed end hollow fibers for bubbleless membrane aeration: experimental studies." Journal of Membrane Science 69(1-2): 1-10.

Akinnawo, S. O. (2023). "Eutrophication: Causes, Consequences, Physical, Chemical and Biological Techniques for Mitigation Strategies." Environmental Challenges: 100733.

Anh-Vu, N., Yun-Je, L., Masumi, K. & Visvanathan, C. 2022. Effects of membrane relaxation rate on performance of pilot-scale membrane aerated biofilm reactors treating domestic wastewater. Environmental Research, 211, 113003.

APHA AWWA, W. (1998). "Standard methods for the examination of water and wastewater 20th edition." American Public Health Association, American Water Work Association, Water Environment Federation, Washington, DC.

Aqeel, H., D. G. Weissbrodt, M. Cerruti, G. M. Wolfaardt, B.-M. Wilén and S. N. Liss (2019). "Drivers of bioaggregation from flocs to biofilms and granular sludge." Environmental Science: Water Research & Technology 5(12): 2072-2089.

Awolusi, O., Kumari, S. & Bux, F. 2015. Ecophysiology of nitrifying communities in membrane bioreactors. International Journal of Environmental Science and Technology, 12, 747-762.

Aybar, M., G. Pizarro, J. Boltz and R. Nerenberg (2015). Energy efficient wastewater treatment via the membrane-aerated biofilm reactor (MABR): Effect of membrane fluxes on performance. WEFTEC 2015, Water Environment Federation.

Aybar, M., G. Pizarro, J. Boltz, L. Downing and R. Nerenberg (2014). "Energy-efficient wastewater treatment via the air-based, hybrid membrane biofilm reactor (hybrid MfBR)." Water science and technology 69(8): 1735-1741.

Bassin, J., Kleerebezem, R., Rosado, A., Van Loosdrecht, M. M. & Dezotti, M. 2012. Effect of different operational conditions on biofilm development, nitrification, and nitrifying microbial population in moving-bed biofilm reactors. Environmental Science & Technology, 46, 1546-1555.

Begmatov, S., A. G. Dorofeev, V. V. Kadnikov, A. V. Beletsky, N. V. Pimenov, N. V. Ravin and A. V. Mardanov (2022). "The structure of microbial communities of activated sludge of large-scale wastewater treatment plants in the city of Moscow." Scientific reports 12(1): 3458.

Bian, X., Y. Wu, J. Li, M. Yin, D. Li, H. Pei, S. Chang and W. Guo (2022). "Effect of dissolved oxygen on high C/N wastewater treatment in moving bed biofilm reactors based on heterotrophic nitrification and aerobic denitrification: Nitrogen removal performance and potential mechanisms." Bioresource Technology 365: 128147.

Blackburne, R., Z. G. Yuan and J. Keller (2008). "Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor." Biodegradation. 19.

Boltz, J. and G. Daigger (2010). "Uncertainty in bulk-liquid hydrodynamics and biofilm dynamics creates uncertainties in biofilm reactor design." Water Science and Technology 61(2): 307-316.

Boltz, J. P., B. F. Smets, B. E. Rittmann, M. C. Van Loosdrecht, E. Morgenroth and G. T. Daigger (2017). "From biofilm ecology to reactors: a focused review." Water Science and Technology 75(8): 1753-1760.

Bunse, P., L. Orschler, S. Agrawal and S. Lackner (2020). "Membrane aerated biofilm reactors for mainstream partial nitritation/anammox: Experiences using real municipal wastewater." Water Research X 9: 100066.

Callahan, B. J., Mcmurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A. & Holmes, S. P. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature methods, 13, 581-583.

Canto-Encalada, G., D. Tec-Campos, J. D. Tibocha-Bonilla, K. Zengler, A. Zepeda and C. Zuñiga (2022). "Flux balance analysis of the ammonia-oxidizing bacterium Nitrosomonas europaea ATCC19718 unravels specific metabolic activities while degrading toxic compounds." PLoS Computational Biology 18(2): e1009828.

Cao, C., L. Zhao, D. Xu and Q. Geng (2009). "Membrane-aerated biofilm reactor behaviors for the treatment of high-strength ammonium industrial wastewater." Chemical Engineering & Technology: Industrial Chemistry-Plant Equipment-Process Engineering-Biotechnology 32(4): 613-621.

Carlson, A. L., H. He, C. Yang and G. T. Daigger (2021). "Comparison of hybrid membrane aerated biofilm reactor (MABR)/suspended growth and conventional biological nutrient removal processes." Water Science and Technology 83(6): 1418-1428.

Carniello, V., B. W. Peterson, H. C. van der Mei and H. J. Busscher (2018). "Physico-chemistry from initial bacterial adhesion to surface-programmed biofilm growth." Advances in colloid and interface science 261: 1-14.

Casey, E., B. Glennon and G. Hamer (1999). "Oxygen mass transfer characteristics in a membrane-aerated biofilm reactor." Biotechnology and bioengineering 62(2): 183-192.

Casey, E., B. Glennon and G. Hamer (2000). "Biofilm development in a membrane-aerated biofilm reactor: Effect of flow velocity on performance." Biotechnology and Bioengineering 67(4): 476-486.

Castagna, L., A. Zanella, V. Scaravilli, F. Magni, S. A. E. A. E. S. Deab, M. Introna, F. Mojoli, G. Grasselli, A. Pesenti and N. Patroniti (2015). "Effects on membrane lung gas exchange of an intermittent high gas flow recruitment maneuver: preliminary data in veno-venous ECMO patients." Journal of Artificial Organs 18: 213-219.

Castrillo, M., R. Díez-Montero, A. L. Esteban-García and I. Tejero (2019). "Mass transfer enhancement and improved nitrification in MABR through specific membrane configuration." Water research 152: 1-11.

Chai, H., Y. Xiang, R. Chen, Z. Shao, L. Gu, L. Li and Q. He (2019). "Enhanced simultaneous nitrification and denitrification in treating low carbon-to-nitrogen ratio wastewater: Treatment performance and nitrogen removal pathway." Bioresource technology 280: 51-58.

Chen, R. and Y. Zhou (2021). "Measure microbial activity driven oxygen transfer in membrane aerated biofilm reactor from supply side." Environmental Research 195: 110845.

Chen, R., Cao, S., Zhang, L. & Zhou, Y. 2022. NOB suppression strategies in a mainstream membrane aerated biofilm reactor under exceptionally low lumen pressure. Chemosphere, 290, 133386.

Chen, Y., S. Lan, L. Wang, S. Dong, H. Zhou, Z. Tan and X. Li (2017). "A review: driving factors and regulation strategies of microbial community structure and dynamics in wastewater treatment systems." Chemosphere 174: 173-182.

Choi, M., K. Cho, D. Jeong, Y.-C. Chung, J. Park, S. Lee and H. Bae (2018). "Effects of the ammonium loading rate on nitrite-oxidizing activity during nitrification at a high dose of inorganic carbon." Journal of Environmental Science and Health, Part A 53(8): 708-717.

Cole, A. C., J. W. Shanahan, M. J. Semmens and T. M. LaPara (2002). "Preliminary studies on the microbial community structure of membrane-aerated biofilms treating municipal wastewater." Desalination 146(1-3): 421-426.

Cole, A. C., M. J. Semmens and T. M. LaPara (2004). "Stratification of activity and bacterial community structure in biofilms grown on membranes transferring oxygen." Applied and Environmental Microbiology 70(4): 1982-1989.

Corsino, S. F. and M. Torregrossa (2022). "Achieving complete nitrification below the washout SRT with hybrid membrane aerated biofilm reactor (MABR) treating municipal wastewater." Journal of Environmental Chemical Engineering 10(1): 106983.

Côté, P., J. Peeters, N. Adams, Y. Hong, Z. Long and J. Ireland (2015). A new membrane-aerated biofilm reactor for low energy wastewater treatment: pilot results. WEFTEC 2015, Water Environment Federation.

Cote, P., J.-L. Bersillon, A. Huyard and G. Faup (1988). "Bubble-free aeration using membranes: process analysis." Journal (Water Pollution Control Federation): 1986-1992.

Côté, P., Peeters, J., Adams, N., Hong, Y., Long, Z. & Ireland, J. 2015. A new membrane-aerated biofilm reactor for low energy wastewater treatment: Pilot results. Proceedings of the Water Environment Federation, 13, 4226-4239.

Cydzik-Kwiatkowska, A. & Zielińska, M. 2016. Bacterial communities in full-scale wastewater treatment systems. World Journal of Microbiology and Biotechnology, 32, 1-8.

Czieborowski, M., Hübenthal, A., Poehlein, A., Vogt, I. & Philipp, B. 2020. Genetic and physiological analysis of biofilm formation on different plastic surfaces by Sphingomonas sp. strain S2M10 reveals an essential function of sphingan biosynthesis. Microbiology, 166, 918-935.

Da Silva, T. S., Matsumoto, T., Dos Anjos, M. L. & Albertin, L. L. 2018. Organic matter removal in a membrane-aerated biofilm reactor. Journal of Environmental Engineering, 144, 04018057.

De Meis, D. (2017). Gas transport through porous membranes, ENEA.

De-la-Pinta, I., M. Cobos, J. Ibarretxe, E. Montoya, E. Eraso, T. Guraya and G. Quindós (2019). "Effect of biomaterials hydrophobicity and roughness on biofilm development." Journal of Materials Science: Materials in Medicine 30: 1-11.

Denkhaus, E., S. Meisen, U. Telgheder and J. Wingender (2007). "Chemical and physical methods for characterisation of biofilms." Microchimica Acta 158: 1-27.

Dodds, W. K. & Welch, E. B. 2000. Establishing nutrient criteria in streams. Journal of the North American Benthological Society, 19, 186-196.

Dong, W.-y., H.-j. Wang, W.-g. Li, W.-c. Ying, G.-h. Gan and Y. Yang (2009). "Effect of DO on simultaneous removal of carbon and nitrogen by a membrane aeration/filtration combined bioreactor." Journal of Membrane Science 344(1-2): 219-224.

Downing, L. S. and R. Nerenberg (2008). "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.

Elsayed, A., M. Hurdle and Y. Kim (2021). "Comprehensive model applications for better understanding of pilot-scale membrane-aerated biofilm reactor performance." Journal of Water Process Engineering 40: 101894.

Fitzgerald, C. M., Camejo, P., Oshlag, J. Z. & Noguera, D. R. 2015. Ammonia-oxidizing microbial communities in reactors with efficient nitrification at low-dissolved oxygen. Water research, 70, 38-51.

Frølund, B., Palmgren, R., Keiding, K. & Nielsen, P. H. 1996. Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water research, 30, 1749-1758.

Ge, S., S. Wang, X. Yang, S. Qiu, B. Li and Y. Peng (2015). "Detection of nitrifiers and evaluation of partial nitrification for wastewater treatment: A review." Chemosphere 140: 85-98.

Gilbert, E. M., Agrawal, S., Brunner, F., Schwartz, T., Horn, H. & Lackner, S. 2014. Response of different Nitrospira species to anoxic periods depends on operational DO. Environmental science & technology, 48, 2934-2941.

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

Gude, V. G. (2015). "Energy and water autarky of wastewater treatment and power generation systems." Renewable and sustainable energy reviews 45: 52-68.

Godzieba, M., M. Zubrowska-Sudol, J. Walczak and S. Ciesielski (2022). "Development of microbial communities in biofilm and activated sludge in a hybrid reactor." Scientific Reports 12(1): 12558.

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

Grady Jr, C. L., G. T. Daigger, N. G. Love and C. D. Filipe (2011). Biological wastewater treatment, CRC press.

Guglielmi, G., D. Coutts, D. Houweling and J. Peeters (2020). "FULL-SCALE APPLICATION OF MABR TECHNOLOGY FOR UPGRADING AND RETROFITTING AN EXISTING WWTP: PERFORMANCES AND PROCESS MODELLING." Environmental Engineering & Management Journal (EEMJ) 19(10).

Hall-Stoodley, L., Costerton, J. W. & Stoodley, P. 2004. Bacterial biofilms: from the natural environment to infectious diseases. Nature reviews microbiology, 2, 95-108.

He, H., B. M. Wagner, A. L. Carlson, C. Yang and G. T. Daigger (2021). "Recent progress using membrane aerated biofilm reactors for wastewater treatment." Water Science and Technology 84(9): 2131-2157.

Heffernan, B., A. Shrivastava, D. Toniolo, M. Semmens and E. Syron (2017). Operation of a large scale membrane aerated biofilm reactor for the treatment of municipal wastewater. WEFTEC 2017, Water Environment Federation.

Heffernan, B., A. Shrivastava, D. Toniolo, M. Semmens and E. Syron (2017). Operation of a large scale membrane aerated biofilm reactor for the treatment of municipal wastewater. WEFTEC 2017, Water Environment Federation.

Hibiya, K., A. Terada, S. Tsuneda and A. Hirata (2003). "Simultaneous nitrification and denitrification by controlling vertical and horizontal microenvironment in a membrane-aerated biofilm reactor." Journal of biotechnology 100(1): 23-32.

Hoa, P., L. Nair and C. Visvanathan (2003). "The effect of nutrients on extracellular polymeric substance production and its influence on sludge properties." Water Sa 29(4): 437-442.

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

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

Houweling, D. & Daigger, G. T. 2019. Intensifying Activated Sludge Using Media-Supported Biofilms, CRC Press.

Houweling, D., J. Peeters, P. Cote, Z. Long and N. Adams (2017). "Proving membrane aerated biofilm reactor (MABR) performance and reliability: Results from four pilots and a full-scale plant." Proceedings of the Water Environment Federation 2017(16): 272-284.

Hu, S., F. Yang, S. Liu and L. Yu (2009). "The development of a novel hybrid aerating membrane-anaerobic baffled reactor for the simultaneous nitrogen and organic carbon removal from wastewater." Water Research 43(2): 381-388.

Hu, X. b., K. Xu, Z. Wang, L. l. Ding and H. q. Ren (2013). "Characteristics of biofilm attaching to carriers in moving bed biofilm reactor used to treat vitamin C wastewater." Scanning 35(5): 283-291.

Hu, Z., D. Houweling and P. Dold (2012). "Biological nutrient removal in municipal wastewater treatment: new directions in sustainability." Journal of Environmental Engineering 138(3): 307-317.

Hwang, J. H., Cicek, N. & Oleszkiewicz, J. A. 2009. Membrane biofilm reactors for nitrogen removal: state-of-the-art and research needs. Water Science and Technology, 60, 2739-2747.

Iorhemen, O. T., S. Ukaigwe, H. Dang and Y. Liu (2022). "Phosphorus Removal from Aerobic Granular Sludge: Proliferation of Polyphosphate-Accumulating Organisms (PAOs) under Different Feeding Strategies." Processes 10(7): 1399.

Janczewski, L. & Trusek-Holownia, A. 2016. Biofilm-based membrane reactors–selected aspects of the application and microbial layer control. Desalination and Water Treatment, 57, 22909-22916.

Kelly, J. J., London, M. G., Mccormick, A. R., Rojas, M., Scott, J. W. & Hoellein, T. J. 2021. Wastewater treatment alters microbial colonization of microplastics. PloS one, 16, e0244443.

Khalid, S., Shahid, M., Bibi, I., Sarwar, T., Shah, A. H. & Niazi, N. K. 2018. A review of environmental contamination and health risk assessment of wastewater use for crop irrigation with a focus on low and high-income countries. International journal of environmental research and public health, 15, 895.

Kinh, C. T., S. Riya, M. Hosomi and A. Terada (2017). "Identification of hotspots for NO and N2O production and consumption in counter-and co-diffusion biofilms for simultaneous nitrification and denitrification." Bioresource technology 245: 318-324.

Kitzinger, K., H. Koch, S. Lücker, C. J. Sedlacek, C. Herbold, J. Schwarz, A. Daebeler, A. J. Mueller, M. Lukumbuzya and S. Romano (2018). "Characterization of the first “Candidatus Nitrotoga” isolate reveals metabolic versatility and separate evolution of widespread nitrite-oxidizing bacteria." MBio 9(4).

Krzeminski, P., L. Leverette, S. Malamis and E. Katsou (2017). "Membrane bioreactors–a review on recent developments in energy reduction, fouling control, novel configurations, LCA and market prospects." Journal of Membrane Science 527: 207-227.

Koch, H., S. Lücker, M. Albertsen, K. Kitzinger, C. Herbold, E. Spieck, P. H. Nielsen, M. Wagner and H. Daims (2015). "Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus Nitrospira." Proceedings of the national academy of sciences 112(36): 11371-11376.

Koskinen, R., T. Ali-Vehmas, P. Kämpfer, M. Laurikkala, I. Tsitko, E. Kostyal, F. Atroshi and M. Salkinoja-Salonen (2000). "Characterization of Sphingomonas isolates from Finnish and Swedish drinking water distribution systems." Journal of Applied Microbiology 89(4): 687-696.

Kostera, J., M. D. Youngblut, J. M. Slosarczyk and A. A. Pacheco (2008). "Kinetic and product distribution analysis of NO· reductase activity in Nitrosomonas europaea hydroxylamine oxidoreductase." JBIC Journal of Biological Inorganic Chemistry 13: 1073-1083.

Krause, S., B. Zimmermann, U. Meyer-Blumenroth, W. Lamparter, B. Siembida and P. Cornel (2010). "Enhanced membrane bioreactor process without chemical cleaning." Water Science and Technology 61(10): 2575-2580.

Krishna, K. B., A. Sathasivan and M. P. Ginige (2013). "Microbial community changes with decaying chloramine residuals in a lab-scale system." Water research 47(13): 4666-4679.

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

Kurisu, F., F. Kurisu, Y. Sakamoto and O. Yagi (2007). "Effects of ammonium and nitrite on communities and populations of ammonia-oxidizing bacteria in laboratory-scale continuous-flow reactors." FEMS microbiology ecology 60(3): 501-512.

Lackner, S., A. Terada, H. Horn, M. Henze and B. F. Smets (2010). "Nitritation performance in membrane-aerated biofilm reactors differs from conventional biofilm systems." Water Research 44(20): 6073-6084.

Lai, C.-Y., L. Zhong, Y. Zhang, J.-X. Chen, L.-L. Wen, L.-D. Shi, Y.-P. Sun, F. Ma, B. E. Rittmann and C. Zhou (2016). "Bioreduction of chromate in a methane-based membrane biofilm reactor." Environmental science & technology 50(11): 5832-5839.

LaPara, T. M., A. C. Cole, J. W. Shanahan and M. J. Semmens (2006). "The effects of organic carbon, ammoniacal-nitrogen, and oxygen partial pressure on the stratification of membrane-aerated biofilms." Journal of Industrial Microbiology and Biotechnology 33(4): 315-323.

Latocheski, E. C., M. C. V. da Rocha and M. C. B. Braga (2022). "Nitrospira in wastewater treatment: applications, opportunities and research gaps." Reviews in Environmental Science and Bio/Technology 21(4): 905-930.

Lawson, C. E. and S. Lücker (2018). "Complete ammonia oxidation: an important control on nitrification in engineered ecosystems?" Current opinion in biotechnology 50: 158-165.

Lee, H. S. and B. Liao (2021). "Anaerobic membrane bioreactors for wastewater treatment: Challenges and opportunities." Water Environment Research 93(7): 993-1004.

Lema, J. M. and S. S. Martinez (2017). Innovative wastewater treatment & resource recovery technologies: impacts on energy, economy and environment, IWA publishing.

Li, J., L.-P. Zhu, Y.-Y. Xu and B.-K. Zhu (2010). "Oxygen transfer characteristics of hydrophilic treated polypropylene hollow fiber membranes for bubbleless aeration." Journal of Membrane Science 362(1-2): 47-57.

Li, M., C. Du, J. Liu, X. Quan, M. Lan and B. Li (2018). "Mathematical modeling on the nitrogen removal inside the membrane-aerated biofilm dominated by ammonia-oxidizing archaea (AOA): effects of temperature, aeration pressure and COD/N ratio." Chemical Engineering Journal 338: 680-687.

Li, M., Y. Li, N. Wang, T. Li, H. Guo, Z. Wu, P. Zhang, B. Wang and B. Li (2022). "Achieving efficient nitrogen removal in a single-stage partial nitrification-anammox-partial denitrification (PN/A/PD) membrane aerated biofilm reactor (MABR)." Journal of Water Process Engineering 49: 103100.

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

Li, X., Bao, D., Zhang, Y., Xu, W., Zhang, C., Yang, H., Ru, Q., Wang, Y.-F., Ma, H. & Zhu, E. 2023. Development and application of membrane aerated biofilm reactor (MABR)—A review. Water, 15, 436.

Li, X., D. Bao, Y. Zhang, W. Xu, C. Zhang, H. Yang, Q. Ru, Y.-f. Wang, H. Ma and E. Zhu (2023). "Development and application of membrane aerated biofilm reactor (MABR)—A review." Water 15(3): 436.

Li, Y. and K. Zhang (2018). "Pilot scale treatment of polluted surface waters using membrane-aerated biofilm reactor (MABR)." Biotechnology & Biotechnological Equipment 32(2): 376-386.

Liang, Z., Li, W., Yang, S. & Du, P. 2010. Extraction and structural characteristics of extracellular polymeric substances (EPS), pellets in autotrophic nitrifying biofilm and activated sludge. Chemosphere, 81, 626-632.

Liao, B. and S. Liss (2007). "A comparative study between thermophilic and mesophilic membrane aerated biofilm reactors." Journal of Environmental Engineering and Science 6(2): 247-252.

Limpiyakorn, T., Y. Shinohara, F. Kurisu and O. Yagi (2005). "Communities of ammonia-oxidizing bacteria in activated sludge of various sewage treatment plants in Tokyo." FEMS microbiology ecology 54(2): 205-217.

Lin, J., P. Zhang, G. Li, J. Yin, J. Li and X. Zhao (2016). "Effect of COD/N ratio on nitrogen removal in a membrane-aerated biofilm reactor." International Biodeterioration & Biodegradation 113: 74-79.

Lin, J., P. Zhang, J. Yin, X. Zhao and J. Li (2015). "Nitrogen removal performances of a polyvinylidene fluoride membrane-aerated biofilm reactor." International Biodeterioration & Biodegradation 102: 49-55.

Lin, J., Zhang, P., Li, G., Yin, J., Li, J. & Zhao, X. 2016. Effect of COD/N ratio on nitrogen removal in a membrane-aerated biofilm reactor. International Biodeterioration & Biodegradation, 113, 74-79.

Liu, Y., J. Gu and M. Zhang (2019). AB processes: Towards energy self-sufficient municipal wastewater treatment, IWA publishing.

Liu, G. & Wang, J. 2013. Long-term low DO enriches and shifts nitrifier community in activated sludge. Environmental science & technology, 47, 5109-5117.

Liu, H., F. Yang, T. Wang, Q. Liu and S. Hu (2007). "Carbon membrane-aerated biofilm reactor for synthetic wastewater treatment." Bioprocess and biosystems engineering 30(4): 217-224.

Liu, H., S. Tan, Z. Sheng, Y. Liu and T. Yu (2014). "Bacterial community structure and activity of sulfate reducing bacteria in a membrane aerated biofilm analyzed by microsensor and molecular techniques." Biotechnology and Bioengineering 111(11): 2155-2162.

Liu, W., Q. Yang, B. Ma, J. Li, L. Ma, S. Wang and Y. Peng (2017). "Rapid achievement of nitritation using aerobic starvation." Environmental Science & Technology 51(7): 4001-4008.

Liu, W., W. Chen, D. Yang and Y. Shen (2019). "Functional and compositional characteristics of nitrifiers reveal the failure of achieving mainstream nitritation under limited oxygen or ammonia conditions." Bioresource technology 275: 272-279.

Liu, X., M. Kim, G. Nakhla, M. Andalib and Y. Fang (2020). "Partial nitrification-reactor configurations, and operational conditions: Performance analysis." Journal of Environmental Chemical Engineering 8(4): 103984.

Liu, Y., H. H. Ngo, W. Guo, L. Peng, D. Wang and B. Ni (2019). "The roles of free ammonia (FA) in biological wastewater treatment processes: A review." Environment international 123: 10-19.

Long, Z., A. K. Oskouie, T. E. Kunetz, J. Peeters, N. Adams and D. Houweling (2020). "Simulation of long-term performance of an innovative membrane-aerated biofilm reactor." Journal of Environmental Engineering 146(6): 04020041.

Lu, D., H. Bai, F. Kong, S. N. Liss and B. Liao (2021). "Recent advances in membrane aerated biofilm reactors." Critical Reviews in Environmental Science and Technology 51(7): 649-703.

Lu, J.-J., Q. Shen, X.-Y. Li, F.-Y. Sun, J.-B. Yi, W.-Y. Dong, W.-J. Yan, H. Du and J.-L. Mu (2022). "Surface-manipulated membranes to accelerate biofilm formation and to resist bacterial detachment in MBfR for aerobic methane oxidation coupled to denitrification." Chemical Engineering Journal 430: 132629.

Luo, Y.-H., Chen, R., Wen, L.-L., Meng, F., Zhang, Y., Lai, C.-Y., Rittmann, B. E., Zhao, H.-P. & Zheng, P. 2015. Complete perchlorate reduction using methane as the sole electron donor and carbon source. Environmental science & technology, 49, 2341-2349.

Ma, Y., A. Piscedda, A. D. L. C. Veras, C. Domingo-Félez and B. F. Smets (2022). "Intermittent aeration to regulate microbial activities in membrane-aerated biofilm reactors: Energy-efficient nitrogen removal and low nitrous oxide emission." Chemical Engineering Journal 433: 133630.

Ma, Y., C. Domingo-Felez, B. G. Plósz and B. F. Smets (2017). "Intermittent aeration suppresses nitrite-oxidizing bacteria in membrane-aerated biofilms: a model-based explanation." Environmental science & technology 51(11): 6146-6155.

Ma, Y., Piscedda, A., Veras, A. D. L. C., Domingo-Félez, C. & Smets, B. F. 2022. Intermittent aeration to regulate microbial activities in membrane-aerated biofilm reactors: Energy-efficient nitrogen removal and low nitrous oxide emission. Chemical Engineering Journal, 433, 133630.

Mahmood, Z., H. Cheng and M. Tian (2022). "A critical review on advanced anaerobic membrane bioreactors (AnMBR) for wastewater treatment: advanced membrane materials and energy demand." Environmental Science: Water Research & Technology.

Maddela, N. R., Z. Gan, Y. Meng, F. Fan and F. Meng (2022). "Occurrence and roles of comammox bacteria in water and wastewater treatment systems: a critical review." Engineering 17: 196-206.

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

Martin, K. J., J. P. Boltz and R. Nerenberg (2012). The Membrane Biofilm Reactor (MBfR) for Wastewater Treatment: Applications, Design Considerations, and Technology Outlook. Proceedings of 85th Annual Water Environment Federation Technical Exposition & Conference.

Mccarty, P. L., Bae, J. & Kim, J. 2011. Domestic wastewater treatment as a net energy producer–can this be achieved? : ACS Publications.

McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight and P. Hugenholtz (2012). "An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea." The ISME journal 6(3): 610-618.

Mcdonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., Desantis, T. Z., Probst, A., Andersen, G. L., Knight, R. & Hugenholtz, P. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME journal, 6, 610-618.

Mehrabi, S., D. Houweling and M. Dagnew (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.

Melo, L. F. (2003). "Biofilm formation and its role in fixed film processes." book: Water Wastewater Microbiology: 337-349.

Metcalf, Eddy, M. Abu-Orf, G. Bowden, F. L. Burton, W. Pfrang, H. D. Stensel, G. Tchobanoglous, R. Tsuchihashi and AECOM (2014). Wastewater engineering: treatment and resource recovery, McGraw Hill Education.

Miao, Y., L. Zhang, Y. Yang, Y. Peng, B. Li, S. Wang and Q. Zhang (2016). "Start-up of single-stage partial nitrification-anammox process treating low-strength swage and its restoration from nitrate accumulation." Bioresource Technology 218: 771-779.

Mielczarek, A. T., H. T. T. Nguyen, J. L. Nielsen and P. H. Nielsen (2013). "Population dynamics of bacteria involved in enhanced biological phosphorus removal in Danish wastewater treatment plants." Water research 47(4): 1529-1544.

Ming, T., R. De_richter, S. Shen and S. Caillol (2016). "Fighting global warming by greenhouse gas removal: destroying atmospheric nitrous oxide thanks to synergies between two breakthrough technologies." Environmental Science and Pollution Research 23: 6119-6138.

Mohan, S. M. and S. Nagalakshmi (2020). "A review on aerobic self-forming dynamic membrane bioreactor: Formation, performance, fouling and cleaning." Journal of Water Process Engineering 37: 101541.

Mota, C., J. Ridenoure, J. Cheng and F. L. de los Reyes III (2005). "High levels of nitrifying bacteria in intermittently aerated reactors treating high ammonia wastewater." FEMS Microbiology Ecology 54(3): 391-400.

Nancharaiah, Y. V. and M. Sarvajith (2019). "Aerobic granular sludge process: a fast-growing biological treatment for sustainable wastewater treatment." Current Opinion in Environmental Science & Health 12: 57-65.

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

Nerenberg, R. (2005). Membrane biofilm reactors for water and wastewater treatment. Borchardt Conference: A Seminar on Advances in Water and Wastewater Treatment, Ann Arbor. MI.

Nerenberg, R. (2005). Membrane biofilm reactors for water and wastewater treatment. Borchardt Conference: A Seminar on Advances in Water and Wastewater Treatment, Ann Arbor. MI.

Nerenberg, R. (2016). "The membrane-biofilm reactor (MBfR) as a counter-diffusional biofilm process." Current opinion in biotechnology 38: 131-136.

Nisola, G. M., J. Orata-Flor, S. Oh, N. Yoo and W.-J. Chung (2013). "Partial nitrification in a membrane-aerated biofilm reactor with composite PEBA/PVDF hollow fibers." Desalination and Water Treatment 51(25-27): 5275-5282.

Nisola, G. M., J. Orata-Flor, S. Oh, N. Yoo and W.-J. Chung (2013). "Partial nitrification in a membrane-aerated biofilm reactor with composite PEBA/PVDF hollow fibers." Desalination and Water Treatment 51(25-27): 5275-5282.

Ohandja, D.-G. and D. C. Stuckey (2007). "Biodegradation of PCE in a hybrid membrane aerated biofilm reactor." Journal of Environmental Engineering 133(1): 20-27.

Okabe, S., Y. Aoi, H. Satoh and Y. Suwa (2011). "Nitrification in wastewater treatment." Nitrification: 405-433.

Ontiveros-Valencia, A., C. Zhou, H.-P. Zhao, R. Krajmalnik-Brown, Y. Tang and B. Rittmann (2018). "Managing microbial communities in membrane biofilm reactors." Applied microbiology and biotechnology 102: 9003-9014.

Pankhania, M., T. Stephenson and M. J. Semmens (1994). "Hollow fibre bioreactor for wastewater treatment using bubbleless membrane aeration." Water research 28(10): 2233-2236.

Park, S., J. Chung, B. E. Rittmann and W. Bae (2015). "Nitrite accumulation from simultaneous free-ammonia and free-nitrous-acid inhibition and oxygen limitation in a continuous-flow biofilm reactor." Biotechnology and Bioengineering 112(1): 43-52.

Park, S., W. Bae and B. E. Rittmann (2010). "Operational boundaries for nitrite accumulation in nitrification based on minimum/maximum substrate concentrations that include effects of oxygen limitation, pH, and free ammonia and free nitrous acid inhibition." Environmental science & technology 44(1): 335-342.

Paśmionka, I. B., K. Bulski, P. Herbut, E. Boligłowa, F. M. C. Vieira, G. Bonassa, M. Bortoli and M. C. d. Prá (2021). "Toxic effect of ammonium nitrogen on the nitrification process and acclimatisation of nitrifying bacteria to high concentrations of NH4-N in wastewater." Energies 14(17): 5329.

Pathak, S., S. Wang and E. Janka (2022). "Achieving Partial Nitritation in Anammox Start-Up Environment." Water 14(2): 229.

Paul, E., J. C. Ochoa, Y. Pechaud, Y. Liu and A. Liné (2012). "Effect of shear stress and growth conditions on detachment and physical properties of biofilms." Water research 46(17): 5499-5508.

Peeters, J., Long, Z., Houweling, D., Côté, P., Daigger, G. T. & Snowling, S. Nutrient removal intensification with MABR–developing a process model supported by piloting. Nutrient Removal and Recovery Symposium 2017, 2017. Water Environment Federation.

Pellicer-Nàcher, C. & Smets, B. F. 2014. Structure, composition, and strength of nitrifying membrane-aerated biofilms. water research, 57, 151-161.

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

Peng, Y. and G. Zhu (2006). "Biological nitrogen removal with nitrification and denitrification via nitrite pathway." Applied microbiology and biotechnology 73: 15-26.

Pérez-Calleja, P., Clements, E. & Nerenberg, R. 2022. Enhancing ammonium oxidation fluxes and nitritation efficiencies in MABRs: a modeling study. Environmental Science: Water Research & Technology, 8, 358-374.

Perez-Calleja, P., M. Aybar, C. Picioreanu, A. L. Esteban-Garcia, K. J. Martin and R. Nerenberg (2017). "Periodic venting of MABR lumen allows high removal rates and high gas-transfer efficiencies." Water research 121: 349-360.

Picioreanu, C., M. C. Van Loosdrecht and J. J. Heijnen (2001). "Two-dimensional model of biofilm detachment caused by internal stress from liquid flow." Biotechnology and bioengineering 72(2): 205-218.

Pierce, J. and C. Suelter (1977). "An evaluation of the Coomassie brilliant blue G-250 dye-binding method for quantitative protein determination." Analytical Biochemistry 81(2): 478-480.

Preisner, M. (2020). "Surface water pollution by untreated municipal wastewater discharge due to a sewer failure." Environmental Processes 7(3): 767-780.

Prosser, J. I., I. M. Head and L. Y. Stein (2014). The family nitrosomonadaceae. The prokaryotes: alphaproteobacteria and betaproteobacteria, Springer Berlin/Heidelberg: 901-918.

Qadir, M., P. Drechsel, B. Jiménez Cisneros, Y. Kim, A. Pramanik, P. Mehta and O. Olaniyan (2020). Global and regional potential of wastewater as a water, nutrient and energy source. Natural resources forum, Wiley Online Library.

Ravishankar, H., A. Nemeth, G. Massons, D. Puig, D. Zardoya, N. Carpi, P. N. Lens and B. Heffernan (2022). "Factors impacting simultaneous nitrification and denitrification in a membrane aerated biofilm reactor (MABR) system treating municipal wastewater." Journal of Environmental Chemical Engineering 10(5): 108120.

Regmi, P., M. W. Miller, B. Holgate, R. Bunce, H. Park, K. Chandran, B. Wett, S. Murthy and C. B. Bott (2014). "Control of aeration, aerobic SRT and COD input for mainstream nitritation/denitritation." Water research 57: 162-171.

Rittmann, B. E. and P. L. McCarty (1980). "Model of steady-state-biofilm kinetics." Biotechnology and bioengineering 22(11): 2343-2357.

Rothemund, C., A. K. Camper and P. Wilderer (1994). "Biofilms growing on gas permeable membranes."

Rout, P. R., M. K. Shahid, R. R. Dash, P. Bhunia, D. Liu, S. Varjani, T. C. Zhang and R. Y. Surampalli (2021). "Nutrient removal from domestic wastewater: A comprehensive review on conventional and advanced technologies." Journal of Environmental Management 296: 113246.

Saghafi, S., G. R. Nabi Bid Hendy and H. Aminirad (2016). "Estimating the electrical energy in different processes for Nasir Abad industrial wastewater treatment plant with emphasis on COD removal." Journal of Environmental Studies 42(1): 19-31.

Salbitani, G. and S. Carfagna (2021). "Ammonium utilization in microalgae: A sustainable method for wastewater treatment." Sustainability 13(2): 956.

Sanchez-Huerta, C., Fortunato, L., Leiknes, T. & Hong, P.-Y. 2022. Influence of biofilm thickness on the removal of thirteen different organic micropollutants via a Membrane Aerated Biofilm Reactor (MABR). Journal of Hazardous Materials, 432, 128698.

Sanchez-Huerta, C., L. Fortunato, T. Leiknes and P.-Y. Hong (2022). "Influence of biofilm

Scherson, Y. D., G. F. Wells, S.-G. Woo, J. Lee, J. Park, B. J. Cantwell and C. S. Criddle (2013). "Nitrogen removal with energy recovery through N 2 O decomposition." Energy & Environmental Science 6(1): 241-248.

Schramm, A., D. De Beer, A. Gieseke and R. Amann (2000). "Microenvironments and distribution of nitrifying bacteria in a membrane-bound biofilm." Environmental microbiology 2(6): 680-686.

Sehar, S. and I. Naz (2016). "Role of the biofilms in wastewater treatment." Microbial biofilms-importance and applications: 121-144.

Semmens, M. and D. Hanus (1999). "Studies of a membrane aerated bioreactor for wastewater treatment." Membrane Technology 1999(111): 9-13.

Semmens, M. and J. Shanahan (2005). "Membrane Technology: Pilot Studies of Membrane Aerated Bioreactors, Final Report." Water Environment Research Foundation, Virginia, USA.

Semmens, M. J., Dahm, K., Shanahan, J. & Christianson, A. 2003. COD and nitrogen removal by biofilms growing on gas permeable membranes. Water Research, 37, 4343-4350.

Shanahan, J. and M. Semmens (2006). "Influence of a nitrifying biofilm on local oxygen fluxes across a micro-porous flat sheet membrane." Journal of Membrane Science 277(1-2): 65-74.

Shanahan, J. W. and M. J. Semmens (2015). "Alkalinity and pH effects on nitrification in a membrane aerated bioreactor: an experimental and model analysis." Water Research 74: 10-22.

Shaowei, H., Fenglin, Y., Cui, S., Zhang, J. & Tonghua, W. 2008. Simultaneous removal of COD and nitrogen using a novel carbon-membrane aerated biofilm reactor. Journal of Environmental Sciences, 20, 142-148.

Sharif Shourjeh, M., P. Kowal, X. Lu, L. Xie and J. Drewnowski (2021). "Development of strategies for AOB and NOB competition supported by mathematical modeling in terms of successful deammonification implementation for energy-efficient WWTPs." Processes 9(3): 562.

Shi, Y., Huang, J., Zeng, G., Gu, Y., Chen, Y., Hu, Y., Tang, B., Zhou, J., Yang, Y. & Shi, L. 2017. Exploiting extracellular polymeric substances (EPS) controlling strategies for performance enhancement of biological wastewater treatments: an overview. Chemosphere, 180, 396-411.

Shinoda, Y., Y. Sakai, H. Uenishi, Y. Uchihashi, A. Hiraishi, H. Yukawa, H. Yurimoto and N. Kato (2004). "Aerobic and anaerobic toluene degradation by a newly isolated denitrifying bacterium, Thauera sp. strain DNT-1." Applied and Environmental Microbiology 70(3): 1385-1392.

Shoji, T., R. Itoh, T. Nittami, T. Kageyama, M. Noguchi and A. Yamasaki (2020). "Influence of the flow velocity on membrane-aerated biofilm reactors: application of a rotating disk for local flow control." Biochemical Engineering Journal 164: 107771.

Siatou, A., A. Manali and P. Gikas (2020). "Energy consumption and internal distribution in activated sludge wastewater treatment plants of Greece." Water **12**(4): 1204.

Silveira, I. T., K. Cadee and W. Bagg (2022). "Startup and initial operation of an MLE-MABR treating municipal wastewater." Water Science and Technology 85(4): 1155-1166.

Siriweera, W. B. S., Yun-Je, L., Masumi, K. & Visvanathan, C. 2021. Organic matter and total nitrogen removal from wastewater using a pilot-scale membrane-aerated biofilm reactor. Bioresource Technology Reports, 15, 100817.

Sliekers, A. O., S. C. Haaijer, M. H. Stafsnes, J. G. Kuenen and M. S. Jetten (2005). "Competition and coexistence of aerobic ammonium-and nitrite-oxidizing bacteria at low oxygen concentrations." Applied microbiology and biotechnology 68: 808-817.

Spasov, E., J. M. Tsuji, L. A. Hug, A. C. Doxey, L. A. Sauder, W. J. Parker and J. D. Neufeld (2020). "High functional diversity among Nitrospira populations that dominate rotating biological contactor microbial communities in a municipal wastewater treatment plant." The ISME Journal 14(7): 1857-1872.

Spieck, E., S. Keuter, T. Wenzel, E. Bock and W. Ludwig (2014). "Characterization of a new marine nitrite oxidizing bacterium, Nitrospina watsonii sp. nov., a member of the newly proposed phylum “Nitrospinae”." Systematic and applied microbiology 37(3): 170-176.

Stricker, A. E., H. Lossing, J. H. Gibson, Y. Hong and J. C. Urbanic (2011). "Pilot Scale Testing of A New Configuration of The Membrane Aerated Biofilm Reactor (MABR) to Treat High-Strength Industrial Sewage." Water Environment Research 83(1): 3-14.

Suarez, C., M. Piculell, O. Modin, S. Langenheder, F. Persson and M. Hermansson (2019). "Thickness determines microbial community structure and function in nitrifying biofilms via deterministic assembly." Scientific reports 9(1): 1-10.

Sun, L., Z. Wang, X. Wei, P. Li, H. Zhang, M. Li, B. Li and S. Wang (2015). "Enhanced biological nitrogen and phosphorus removal using sequencing batch membrane-aerated biofilm reactor." Chemical Engineering Science 135: 559-565.

Sun, S.-P., C. P. i. Nàcher, B. Merkey, Q. Zhou, S.-Q. Xia, D.-H. Yang, J.-H. Sun and B. F. Smets (2010). "Effective biological nitrogen removal treatment processes for domestic wastewaters with low C/N ratios: a review." Environmental Engineering Science 27(2): 111-126.

Sun, Z., Li, Y., Li, M., Wang, N., Liu, J., Guo, H. & Li, B. 2022. Steel pickling rinse wastewater treatment by two-stage MABR system: Reactor performance, extracellular polymeric substances (EPS) and microbial community. Chemosphere, 299, 134402.

Sun, Z., M. Li, G. Wang, X. Yan, Y. Li, M. Lan, R. Liu and B. Li (2020). "Enhanced carbon and nitrogen removal in an integrated anaerobic/anoxic/aerobic-membrane aerated biofilm reactor system." RSC advances 10(48): 28838-28847.

Sunner, N., Z. Long, D. Houweling, A. Monti and J. Peeters (2018). MABR as a low-energy compact solution for nutrient removal upgrades—results from a demonstration in the UK. WEFTEC 2018, Water Environment Federation.

Suzuki, Y., N. Hatano, S. Ito and H. Ikeda (2000). "Performance of nitrogen removal and biofilm structure of porous gas permeable membrane reactor." Water science and technology 41(4-5): 211-217.

Syron, E. & Casey, E. 2008. Membrane-aerated biofilms for high rate biotreatment: performance appraisal, engineering principles, scale-up, and development requirements. Environmental science & technology, 42, 1833-1844.

Syron, E. and B. Heffernan (2017). OxyMem, The Flexiable MABR. Nutrient Removal and Recovery Symposium 2017, Water Environment Federation.

Syron, E., M. J. Semmens and E. Casey (2015). "Performance analysis of a pilot-scale membrane aerated biofilm reactor for the treatment of landfill leachate." Chemical Engineering Journal 273: 120-129.

Taşkan, B., H. Hasar and C. H. Lee (2020). "Effective biofilm control in a membrane biofilm reactor using a quenching bacterium (Rhodococcus sp. BH4)." Biotechnology and Bioengineering 117(4): 1012-1023.

Tay, J.-H., Liu, Q.-S. & Liu, Y. 2002. Aerobic granulation in sequential sludge blanket reactor. Water Science and Technology, 46, 13-18.

Terada, A., Hibiya, K., Nagai, J., Tsuneda, S. & Hirata, A. 2003. Nitrogen removal characteristics and biofilm analysis of a membrane-aerated biofilm reactor applicable to high-strength nitrogenous wastewater treatment. Journal of bioscience and bioengineering, 95, 170-178.

Terada, A., S. Sugawara, T. Yamamoto, S. Zhou, K. Koba and M. Hosomi (2013). "Physiological characteristics of predominant ammonia-oxidizing bacteria enriched from bioreactors with different influent supply regimes." Biochemical engineering journal 79: 153-161.

Terada, A., T. Yamamoto, R. Igarashi, S. Tsuneda and A. Hirata (2006). "Feasibility of a membrane-aerated biofilm reactor to achieve controllable nitrification." Biochemical engineering journal 28(2): 123-130.

Tian, H., H. Zhang, P. Li, L. Sun, F. Hou and B. Li (2015). "Treatment of pharmaceutical wastewater for reuse by coupled membrane-aerated biofilm reactor (MABR) system." RSC advances 5(85): 69829-69838.

Tian, H., M. Hui, P. Pan, J. Huang, L. Chen and J. Zhao (2021). "Performance and microbial ecology of biofilms adhering on aerated membrane with distinctive conditions for the treatment of domestic sewage." Environmental Technology 42(3): 459-467.

Tian, H.-L., J.-Y. Zhao, H.-Y. Zhang, C.-Q. Chi, B.-A. Li and X.-L. Wu (2015). "Bacterial community shift along with the changes in operational conditions in a membrane-aerated biofilm reactor." Applied microbiology and biotechnology 99: 3279-3290.

Timberlake, D. L., S. E. Strand and K. J. Williamson (1988). "Combined aerobic heterotrophic oxidation, nitrification and denitrification in a permeable-support biofilm." Water research 22(12): 1513-1517.

Tirosh, U. and R. Shechter (2020). Membrane aerated biofilm reactor (MABR)—Distributed treatment of wastewater at low energy consumption. Frontiers in Water-Energy-Nexus—Nature-Based Solutions, Advanced Technologies and Best Practices for Environmental Sustainability: Proceedings of the 2nd WaterEnergyNEXUS Conference, November 2018, Salerno, Italy, Springer.

Ukaigwe, S., Y. Zhou, M. Shaheen and Y. Liu (2021). "Municipal wastewater treatment using a membrane aerated biofilm reactor." Journal of Environmental Engineering and Science 17(2): 99-107.

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

Uri-Carreño, N., P. H. Nielsen, K. V. Gernaey and X. Flores-Alsina (2021). "Long-term operation assessment of a full-scale membrane-aerated biofilm reactor under Nordic conditions." Science of the Total Environment 779: 146366.

USEPA, 1999. Wastewater technology fact sheet, fine bubble aeration, EPA 832-F-99-065. U.S. Environmental Protection Agency: EPA Office of Water. Washington DC.

Verstraete, W. and S. E. Vlaeminck (2011). "ZeroWasteWater: short-cycling of wastewater resources for sustainable cities of the future." International Journal of Sustainable Development & World Ecology 18(3): 253-264.

Wagner, B., Daigger, G. & Love, N. 2022. Assessing membrane aerated biofilm reactor configurations in mainstream anammox applications. Water Science and Technology, 85, 943-960.

Wang, B., S. Liu, X. Yao, Y. Liu and G. Qi (2022). "Alternating aeration strategy to reduce aeration energy demand for aerobic granular sludge and analysis of microbial community dynamics." Environmental Science: Water Research & Technology 8(5): 1111-1125.

Wang, H., G. Xu, Z. Qiu, Y. Zhou and Y. Liu (2019). "NOB suppression in pilot-scale mainstream nitritation-denitritation system coupled with MBR for municipal wastewater treatment." Chemosphere 216: 633-639.

Wang, J., J. Liang, L. Sun, J. Shen and M. Wang (2021). "Achieving reliable partial nitrification and anammox process using polyvinyl alcohol gel beads to treat low-strength ammonia wastewater." Bioresource technology 324: 124669.

Wang, R., A. Terada, S. Lackner, B. F. Smets, M. Henze, S. Xia and J. Zhao (2009). "Nitritation performance and biofilm development of co-and counter-diffusion biofilm reactors: modeling and experimental comparison." Water research 43(10): 2699-2709.

Wegen, S., B. Nowka and E. Spieck (2019). "Low temperature and neutral pH define “Candidatus Nitrotoga sp.” as a competitive nitrite oxidizer in coculture with Nitrospira defluvii." Applied and environmental microbiology 85(9).

Werner, J. J., Koren, O., Hugenholtz, P., Desantis, T. Z., Walters, W. A., Caporaso, J. G., Angenent, L. T., Knight, R. & Ley, R. E. 2012. Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys. The ISME journal, 6, 94-103.

Wett, B., A. Omari, S. Podmirseg, M. Han, O. Akintayo, M. Gómez Brandón, S. Murthy, C. Bott, M. Hell and I. Takács (2013). "Going for mainstream deammonification from bench to full scale for maximized resource efficiency." Water science and technology 68(2): 283-289.

Wu, G. & Yin, Q. 2020. Microbial niche nexus sustaining biological wastewater treatment. npj Clean Water, 3, 33.

Wu, Y., Wu, Z., Chu, H., Li, J., Ngo, H. 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.

Xia, S., F. Zhong, Y. Zhang, H. Li and X. Yang (2010). "Bio-reduction of nitrate from groundwater using a hydrogen-based membrane biofilm reactor." Journal of Environmental Sciences 22(2): 257-262.

Xiao, P., J. Zhou, X. Luo, B. Kang, L. Guo, G. Yuan, L. Zhang and T. Zhao (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.

Yamagiwa, K., A. Ohkawa and O. Hirasa (1994). "Simultaneous organic carbon removal and nitrification by biofilm formed on oxygen enrichment membrane." Journal of chemical engineering of Japan 27(5): 638-643.

Yang, Q., Y. Peng, X. Liu, W. Zeng, T. Mino and H. Satoh (2007). "Nitrogen removal via nitrite from municipal wastewater at low temperatures using real-time control to optimize nitrifying communities." Environmental science & technology 41(23): 8159-8164.

Yang, S., S. Xu, A. Mohammed, B. Guo, S. Vincent, N. J. Ashbolt and Y. Liu (2019). "Anammox reactor optimization for the treatment of ammonium rich digestate lagoon supernatant-Step feeding mitigates nitrite inhibition." International biodeterioration & biodegradation 143: 104733.

Yang, S., S. Xu, A. Mohammed, B. Guo, S. Vincent, N. J. Ashbolt and Y. Liu (2019). "Anammox reactor optimization for the treatment of ammonium rich digestate lagoon supernatant-Step feeding mitigates nitrite inhibition." International biodeterioration & biodegradation 143: 104733.

Yeh, S.-J. and C. R. Jenkins (1978). "Pure oxygen fixed film reactor." Journal of the Environmental Engineering Division 104(4): 611-623.

Yim, M.-S., Y. C. Yau, A. Matlow, J.-S. So, J. Zou, C. A. Flemming, H. Schraft and K. T. Leung (2010). "A novel selective growth medium-PCR assay to isolate and detect Sphingomonas in environmental samples." Journal of microbiological methods 82(1): 19-27.

Yin, Q., Sun, Y., Li, B., Feng, Z. & Wu, G. 2022. The r/K selection theory and its application in biological wastewater treatment processes. Science of the Total Environment, 153836.

Zhang, B., Q. Yu, G. Yan, H. Zhu, X. Y. Xu and L. Zhu (2018). "Seasonal bacterial community succession in four typical wastewater treatment plants: correlations between core microbes and process performance." Scientific reports 8(1): 4566.

Zhang, L.-Q., X. Jiang, H.-W. Rong, C.-H. Wei, M. Luo, W.-C. Ma and H.-Y. Ng (2022). "Exploring the carbon and nitrogen removal capacity of a membrane aerated biofilm reactor for low-strength municipal wastewater treatment." Environmental Science: Water Research & Technology 8(2): 280-289.

Zhang, P., B. Feng, Y.-P. Chen, Y.-Z. Dai and J.-S. Guo (2019). "In situ characterizations for EPS-involved microprocesses in biological wastewater treatment systems." Critical Reviews in Environmental Science and Technology 49(11): 917-946.

Zhang, P., J.-S. Guo, Y. Shen, P. Yan, Y.-P. Chen, H. Wang, J.-X. Yang, F. Fang and C. Li (2015). "Microbial communities, extracellular proteomics and polysaccharides: a comparative investigation on biofilm and suspended sludge." Bioresource Technology 190: 21-28.

Zhang, X. and Y. Liu (2022). "Circular economy is game-changing municipal wastewater treatment technology towards energy and carbon neutrality." Chemical Engineering Journal 429: 132114.

Zhao, H., Y. Guo, Q. Wang, Z. Zhang, C. Wu, M. Gao and F. Liu (2022). "The Summary of Nitritation Process in Mainstream Wastewater Treatment." Sustainability 14(24): 16453.

Zheng, M., S. Li, G. Ni, J. Xia, S. Hu, Z. Yuan, Y. Liu and X. Huang (2020). "Critical factors facilitating Candidatus Nitrotoga to be prevalent nitrite-oxidizing bacteria in activated sludge." Environmental science & technology 54(23): 15414-15423.

Zheng, X., S. Zhang, J. Zhang, D. Huang and Z. Zheng (2018). "Advanced nitrogen removal from municipal wastewater treatment plant secondary effluent using a deep bed denitrification filter." Water Science and Technology 77(11): 2723-2732.

Zhou, C., A. Ontiveros-Valencia, R. Nerenberg, Y. Tang, D. Friese, R. Krajmalnik-Brown and B. E. Rittmann (2019). "Hydrogenotrophic microbial reduction of oxyanions with the membrane biofilm reactor." Frontiers in microbiology 9: 3268.

Zhou, S., C. Wei, C. Liao and H. Wu (2010). "Comprehensive study on dynamics of microbial community in Anaerobic-Oxic-Oxic process using PCR-DGGE, gas chromatography analysis, and dehydrogenase activity assays." World Journal of Microbiology and Biotechnology 26: 273-279.

Zhou, Y., Li, R., Guo, B., Xia, S., Liu, Y. & Rittmann, B. E. 2022. The influent COD/N ratio controlled the linear alkylbenzene sulfonate biodegradation and extracellular polymeric substances accumulation in an oxygen-based membrane biofilm reactor. Journal of hazardous materials, 422, 126862.

Zhou, Y., Li, R., Guo, B., Zhang, L., Zou, X., Xia, S. & Liu, Y. 2020. Greywater treatment using an oxygen-based membrane biofilm reactor: Formation of dynamic multifunctional biofilm for organics and nitrogen removal. Chemical Engineering Journal, 386, 123989.

Zhou, Y., P. D. Kiely, R. Kibbee and B. Ormeci (2021). "Effect of polymeric support material on biofilm development, bacterial population, and wastewater treatment performance in anaerobic fixed-film systems." Chemosphere 264: 128477.

Zhu, L., J. Zhou, M. Lv, H. Yu, H. Zhao and X. Xu (2015). "Specific component comparison of extracellular polymeric substances (EPS) in flocs and granular sludge using EEM and SDS-PAGE." Chemosphere 121: 26-32.

Zhuang, H., Z. Wu, L. Xu, S.-Y. Leu and P.-H. Lee (2020). "Energy-efficient single-stage nitrite shunt denitrification with saline sewage through concise dissolved oxygen (DO) supply: process performance and microbial communities." Microorganisms 8(6): 919.

Zou, X., Y. Zhou, M. Gao, S. Yang, A. Mohammed and Y. Liu (2022). "Effective N2O emission control during the nitritation/denitritation treatment of ammonia rich wastewater." Journal of Environmental Chemical Engineering 10(2): 107234.

Appendix

Table A1. Summary of Recent MABR-PND Research Papers

Serial #	Caption	Content brief	Reference
1	NOB suppression strategies in a mainstream membrane aerated biofilm reactor under exceptionally low lumen pressure	Investigated selective NOB suppression strategies in MABR under low (<5 kPa) lumen pressure condition. Cultivating partial nitritation biofilm under zero positive aeration pressure slowed down the growth of NOB.	Chen et al., 2022.
2	Enhancing ammonium oxidation fluxes and nitritation efficiencies in MABRs: a modeling study	Applied 1-D biofilm modeling to explore ammonium oxidation fluxes, (J_{NH4},) and nitritation efficiencies, (η_{NO2}) for MABs and CABs under nitrifying conditions. The work provides a mechanistic understanding of MABR behavior	Pérez-Calleja et al., 2022
3	Membrane aerated biofilm reactors for mainstream partial nitritation/anammox: experiences using real municipal wastewater	Investigated the potential of MABRs for mainstream nitrogen removal via partial nitration/ anammox. Limiting the oxygen supply by intermittent aeration favoured partial nitritation.	*Bunse et al., 2020
4	Establishing mainstream nitrite shunt process in membrane aerated biofilm reactors: Impact of organic carbon and biofilm scouring intensity	Nitrite shunt process in an intermittently aerated MABR was characterized and the impact of sCOD:N ratio and scouring intensity on ammonia and total nitrogen removal efficiencies evaluated.	Mehrabi et al., 2020
5	Intermittent aeration suppresses nitrite-oxidizing bacteria in membrane-aerated biofilms: a model-based explanation	Nitrifying MABR was operated under different intermittent aeration strategies to evaluate nitritation success. 1-D nitrifying biofilm model was developed to explore the potential mechanisms of NOB suppression associated with intermittent aeration. Aeration intermittency and duration were determined to be effective control parameters	Ma et al., 2017
6	Nitrite accumulation from simultaneous free-ammonia and free-nitrous-acid inhibition and oxygen limitation in a continuous-flow biofilm reactor	Multi-species nitrifying biofilm model (MSNBM) was used to identify conditions that should or should not lead to nitrite accumulation. And the effectiveness of each condition was evaluated	Park et al., 2015.
7	Autotrophic nitrogen removal in a membrane-aerated biofilm reactor under continuous aeration: a demonstration.	Nitritation and annamox were successfully coupled in a continuous aerated MABR. Controlling the relative surface loadings of oxygen versus ammonium prevented complete nitrite oxidation and allowed annamox bacteria to develop. Demonstrating that the autotrophic processes can be successfully coupled in an MABR with continuous aeration	*Gilmore et al., 2013.

8	Partial nitrification in a membrane-aerated biofilm reactor with composite PEBA/PVDF hollow fibres.	Two membranes; uncoated PVDF and composite (PEBA 2533) were tested for HF suitability for ammonia removal via nitrite formation in the MABR system. Results reveal that hydrophobic PEBA 2533 was more suitable for bacterial adhesion	Nisola et al., 2013
9	Nitritation performance in membrane-aerated biofilm reactors differs from conventional biofilm systems	MABRs were compared with conventional biofilm reactors to evaluate the influence of environmental conditions and operational parameters on nitritation performance. Crucial nitritation control parameters include; oxygen mass transfer, absolute and relative substrate concentrations in the biofilm.	Lackner et al., 2010
10	Operational boundaries for nitrite accumulation in nitrification based on minimum/maximum substrate concentrations that include effects of oxygen limitation, pH, and free ammonia and free nitrous acid inhibition	Used model to extends the concept of the traditional minimum substrate concentration. Provides a method to identify good combinations of factors that support shortcut nitritation	Park et al., 2010.
11	Multi-species nitrifying biofilm model (MSNBM) including free ammonia and free nitrous acid inhibition and oxygen limitation	Developed MSNBM to address nitrite accumulation by the spatial gradient of pH with biofilm depth and how it induces changes of FA and FNA speciation and inhibition.	Park et al., 2010a.
12	Sequential aeration of membrane-aerated biofilm reactors for high-rate autotrophic nitrogen removal: experimental demonstration.	Two separate MABRs, which displayed limited or no N removal under continuous aeration, removed more than 5.5 g N/m2/day (at loads up to 8 g N/m^2/day) by controlled variation of sequential aeration regimes.	Pellicer-Nàcher et al., 2010
13	Nitritation performance and biofilm development of co-and counter-diffusion biofilm reactors: modelling and experimental comparison.	Comparative study on the start-up performance and biofilm development in two different biofilm reactors with aim of obtaining partial nitritation. Mathematical simulations of the two geometries implemented in two 1-D multispecies biofilm models using the AQUASIM software.	Wang et al., 2009
14	Effect of oxygen gradients on the activity and microbial community structure of a nitrifying, membrane-aerated biofilm	Systematically study of the effects of DO concentration at membrane-biofilm interface on nitrification rates, extent of nitritation and microbial community structure in MABR.	Downing & Nerenberg, 2008
15	Inoculum effects on community composition and nitritation performance of autotrophic nitrifying biofilm reactors with counter-diffusion geometry	Multiple MABRs used to study the connection between microbial community in seeding sludge and nitritation success.	Terada et al. 2010

- * *Process involved anammox*

a) Polyvinylidene fluoride (PVDF)

b) Polyether-block-polyamide copolymer (PEBA)

Figure A.1. Lab-scale membrane aerated biofilm reactor used in the study