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Electronic Supplementary Information

Targeted pressure-based development of membrane biofilms improves anaerobic membrane bioreactor effluent quality

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Table S1 qPCR assessment details: Primers sequences, thermocycling conditions, and amplicon sizes for all genes targeted.

Gene	Primers (5'-3')	Preincubation	Amplification	Cycles	Amplicon (bp)	Reference
sul1	F- CGCACCGGAAACATCGCTGCAC R- TGAAGTTCCGCCGCAAGGCTCG	95°C for 5 min	95°C for 30 s, 55 °C for 30 s, 72°C for 60 s	40	163	(Pei et al., 2006)
sul2	F- TCCGGTGGAGGCCGGTATCTGG R- CGGGAATGCCATCTGCCTTGAG	95°C for 5 min	95°C for 15 s, 56 °C for 30 s, 72℃ for 40s	40	191	(Pei et al., 2006)
tetC	F-GCGGGATATCGTCCATTCCG R-GCGTAGAGGATCCACAGGACG	95°C for 5 min	95°C for 30 s, 55 °C for 30 s, 72°C for 60s	40	207	(Naas et al., 2011)
tetQ	F- AGAATCTGCTGTTTGCCAGTG R- CGGAGTGTCAATGATATTGCA	95°C for 5 min	95°C for 30 s, 55 °C for 30 s, 72°C for 60 s	40	124	(Naas et al., 2011)
ampC	F- CCTCTTGCTCCACATTTGCT R- ACAACGTTTGCTGTGTGACG	95°C for 5 min	95°C for 45 s, 58 °C for 60 s, 72°C for 60s	40	189	(Szczepanowski et al., 2009)
bla_{TEM}	F-TTCCTGTTTTTGCTCACCCAG R-CTCAAGGATCTTACCGCTGTTG	95°C for 5 min	95°C for 45 s, 58 °C for 60 s, 72°C for 60s	40	445	(Bibbal et al., 2007)
intI1	F- CTGGATTTCGATCACGGCACG R- ACATGCGTGTAAATCATCGTCG	95°C for 5 min	95°C for 30 s, 60 °C for 60 s, 72°C for 60s	40	196	(Barlow et al., 2004)
rpoB	F- AACATCGGTTTGATCAAC R- CGTTGCATGTTGGTACCCAT	94°C for 5 min	94°C for 30 s, 50 °C for 90 s, 72°C for 90s	40	381	(Dahllöf et al., 2000)

qPCR methods for gene quantification

The standards were prepared on PCR products. The PCR reactions were conducted using 4 µL of 5x FIREPol Master Mix (Solis BioDyne, USA), 1 µL each of forward and reverse primers at 5 µM, 1 µL DNA template, and 13 µL molecular-grade water, in a 20 µL reaction. PCR products were run on a 1.5 % agarose gel and detected on a ChemiDoc Touching Imaging System (Bio-Rad Laboratories, USA). A sterile scalpel was used to excise bands, which were then purified using the GenElute Gel Extraction Kit (Sigma-Aldrich, USA) according to the manufacturer's protocol. Concentrations of gel extracts were quantified using the AccuGreen™ High Sensitivity dsDNA Quantitation Kit (Biotium) with a Qubit 2.0 Fluorometer (Thermo Fisher, USA). Thermocycling conditions for each primer set used in standard preparation are as shown in Table S2, but with the addition of a final elongation step. For qPCR, each reaction consisted of 20 µL using 10 µL of the Biotium Forget-Me-Not qPCR Master Mix, 1 µL of 5 µM each for forward and reverse primers, 1 µL of the template, and 7 µL of molecular-grade water. Standard curves were generated during qPCR using serial dilutions of the prepared standards at 10^{-2} to 10^{-8} of the stock concentration.

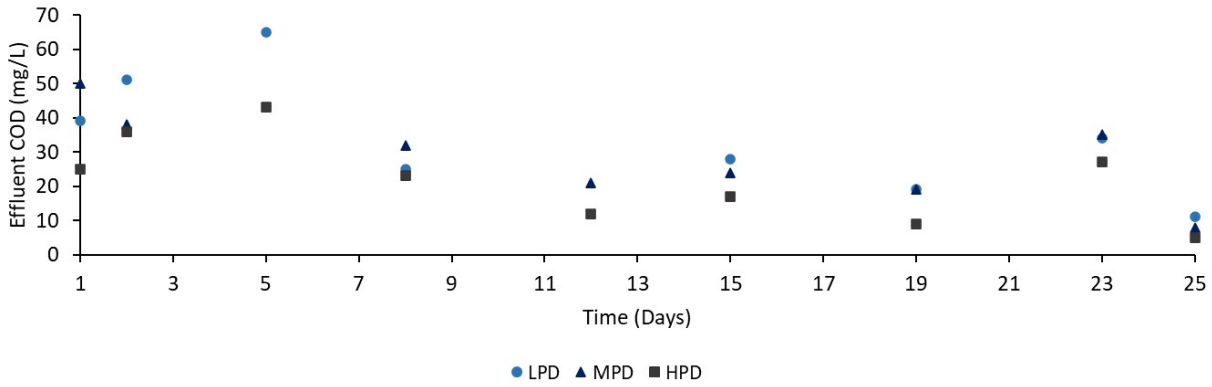

Figure S1 Effluent COD values over course of the experiment from the LPD, MPD, and HPD membranes. Biofilm development was until Day 17 and the operation stage immediately following.

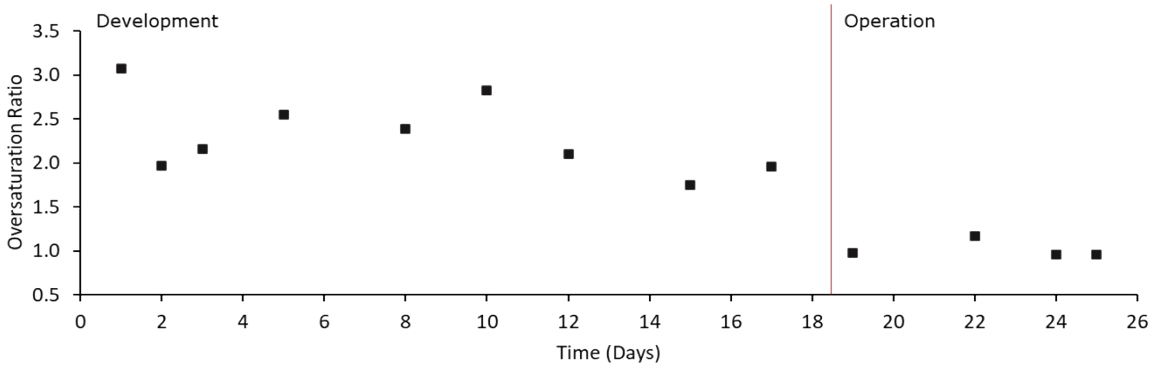

Figure S2 Oversaturation ratio over course of the experiment from the HPD membrane. Biofilm development was until Day 17 and the operation stage immediately following.

Table S2 Representative species with the relative abundance (RA) highest on the HPD membrane – tight layer. LPD and HPD represent the low predeveloped and high predeveloped membrane biofilms, respectively. Loose and tight represent the layers of

Species	LPD - Loose	HPD - Loose	LPD - Tight	HPD - Tight
Azovibrio restrictus (100%)	0.96	0.50	3.94	12.96
Acinetobacter dispersus/ tandoii/ tjernbergiae/haemolyticus/ parvus/ beijerinckii (100%)	0.24	0.22	1.39	6.91
Diaphorobacter polyhydroxybutyrativorans/nitroreducens; Ottowia beijingensis/shaoguanensis/pentelensis ; Comamonas faecalis/serinivorans; Brachymonas chironomi (100%)	1.15	1.99	2.63	4.22
Azospira restricta (97.08%)	1.09	1.62	2.88	4.85
Pseudomonas resinovorans/ guangdongensis (99.6%) or Pseudomonas aeruginosa /otitidis /oryzae/ guezennei (99.21 %)	0.21	0.03	0.57	7.70
Sulfuricurvum kujiense (99.21%)	0.16	0.41	2.40	5.46
Azospira oryzae (100%)	0.01	0.01	1.04	1.56
Acidovorax temperans/defluvii (100%)	0.00	0.01	0.01	2.64
Geobacter lovleyi SZ/thiogenes (100%)	0.64	0.53	1.35	1.64
Rectinema cohabitans (94.86 %)	1.00	0.96	0.76	1.60
Leptolinea tardivitalis (91.67%)	0.95	1.03	0.76	1.43
Klebsiella / Enterobacter / Salmonella species (1005)	0.37	0.71	0.72	1.28
Cloacibacterium normanense/ rupense/ caeni/rupense (100%)	0.00	0.00	0.23	1.35
Psychrobacter sanguinis (100%)	0.01	0.00	0.00	1.17
Thiobacillus sajanensis (100%)	0.01	0.03	0.03	0.94
Moraxella osloensis (1005)	0.00	0.01	0.00	0.63
Aliarcobacter lanthieri/cibarius/ cryaerophilus (99.60%)	0.00	0.00	0.00	0.56
Propionivibrio limicola (98.81 %)	0.00	0.00	0.00	0.55
Clostridium oceanicum (91.73%)	0.08	0.11	0.13	0.55
Desulfovibrio mexicanus (99.60%)	0.05	0.05	0.12	0.50
Novispirillum itersonii species (100%)	0.00	0.00	0.00	0.48
Ruminiclostridium cellobioparum subsp. termitidis /Acetivibrio alkalicellulosi (92.89%)	0.38	0.38	0.38	0.47
Methanothrix / Methanothrix soehngenii (100%)	0.02	0.03	0.07	0.41
Pseudescherichia vulneris/shigella species / Escherichia species (100%)	0.00	0.00	0.00	0.37
Streptococcus minor (99.60%)	0.00	0.01	0.00	0.36
Phascolarctobacterium faecium (85.04%)	0.05	0.05	0.03	0.32
Syntrophomonas zehnderi OL-4 (97.65%)	0.05	0.04	0.06	0.32
Anaerosinus glycerini (100%)	0.02	0.02	0.09	0.18
Actinobacillus seminis (98.81 %)	0.00	0.00	0.00	0.28
Moraxella cuniculi (99.60 %)	0.00	0.00	0.00	0.26
Labilibacter marinus (90.94%)	0.10	0.07	0.02	0.25
Castellaniella caeni / daejeonensis / defragrans / ginsengisoli (100%)	0.00	0.00	0.03	0.04
Luteitalea pratensis (82.75 %)	0.03	0.04	0.03	0.22
Thauera mechernichensis / humireducens (100 %)	0.01	0.00	0.00	0.22
Dialister propionicifaciens / Dialister succinatiphilus YIT 11850 (98.41 %)	0.00	0.00	0.00	0.22
Nitratiruptor tergarcus DSM 16512 (85.10 %)	0.00	0.00	0.00	0.21
Pelistega suis (98.42 %)	0.00	0.01	0.00	0.20

each membrane biofilm

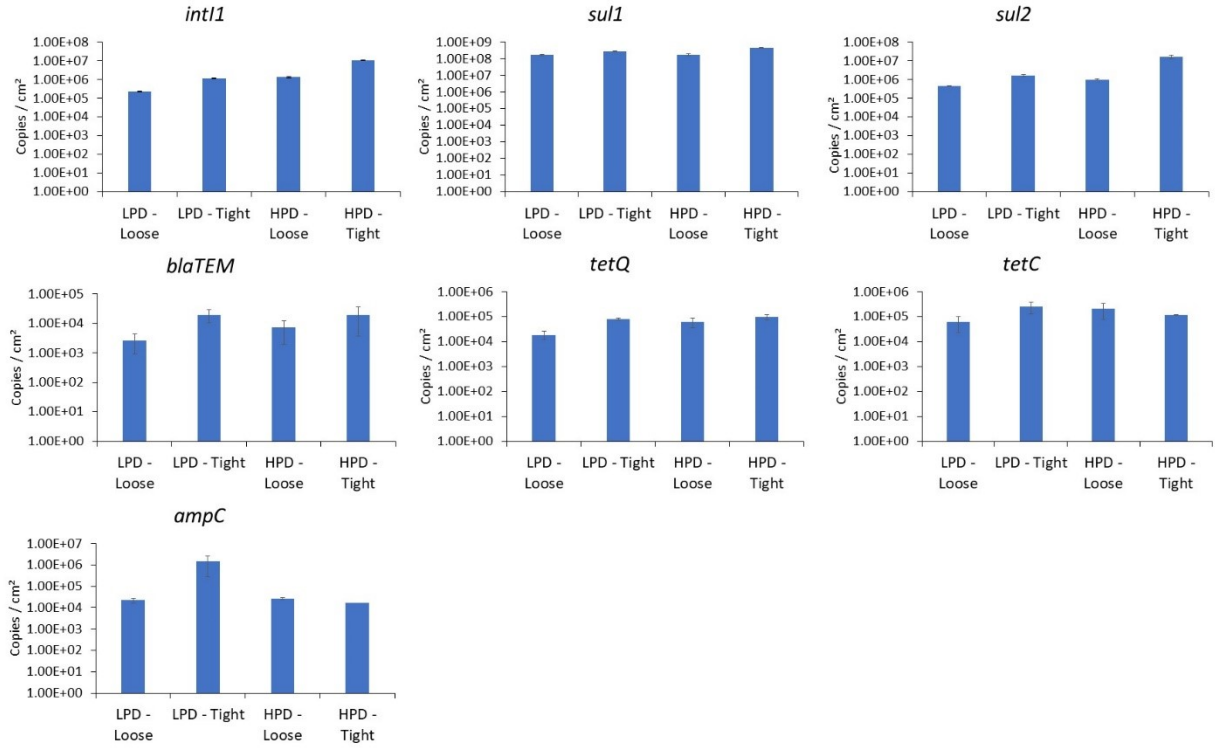

Figure S3 Antibiotic resistance gene (ARG) and intI1 gene copy abundances detected in the AnMBR samples. LPD and HPD represent the Low and high pre-development membrane biofilm, respectively.

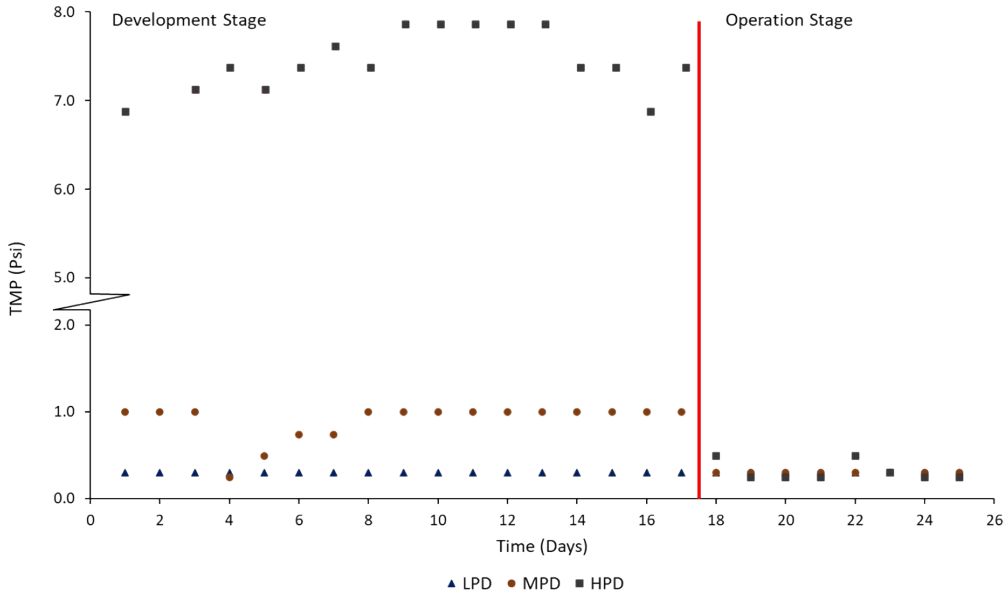

Figure S4 Transmembrane pressure (TMP) during the development and operation stages of the membrane biofilms in the AnMBR. LPD represents low pressure development, MPD medium pressure development, and HPD high pressure development.

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