

Material and Methods

CTAB protocol for DNA extraction:

1. CTAB buffer was prepared with 2% cetyl trimethylammonium bromide (CTAB), 1% polyvinylpyrrolidone, 100 mM Tris-HCl, 1.4 M NaCl and 20 mM EDTA dissolved in double-distilled H₂O (ddH₂O).
2. 1000 µl of CTAB buffer was added to a 2 mL Eppendorf centrifuge tube, then 200 mg of the sludge sample and 0.5 mL of lysozyme buffer (10 mg/mL in water) was added. The mixture was thoroughly mixed by vortex and then kept at 65 °C water bath for 20 minutes.
3. The mixture was centrifuged at 12000 rpm for 10 min. The supernatant was collected and transferred to a new tube. 5 µl of RNase A solution (10 mg/ml in water, DNase-free) and incubate at 37°C for 20 minutes.
4. Then 800 µL of phenol/chloroform/isoamyl alcohol (volume ratio 25:24:1) solution was added. The mixture was mixed by vortex and then centrifuged at 12000 rpm for 10 min.
5. The aqueous phase was collected and transferred to a new tube, then equal volume of chloroform/isoamyl alcohol (volume ratio 24:1) was added. The mixture was mixed by vortex and then centrifuged at 12000 rpm for 10 min.
6. The aqueous phase was collected and transferred to a 1.5 mL tube, then 0.6 volume of isopropanol was added. The mixture was incubated at -20 °C for 30 min to precipitate the DNA.
7. The sample was centrifuged at 12000 rpm for 10 min. The supernatant was

carefully decanted without disturbing the precipitate.

8. The precipitate was washed with 1 mL 75% ethanol for two times.

9. The DNA was dried in clean bench by air to remove the ethanol without completely drying the DNA, otherwise the DNA would be hard to dissolve.

10. The DNA was dissolved with ddH₂O. If necessary, the sample could be incubated at 55-60 °C to dissolve.

Figures and Schemes

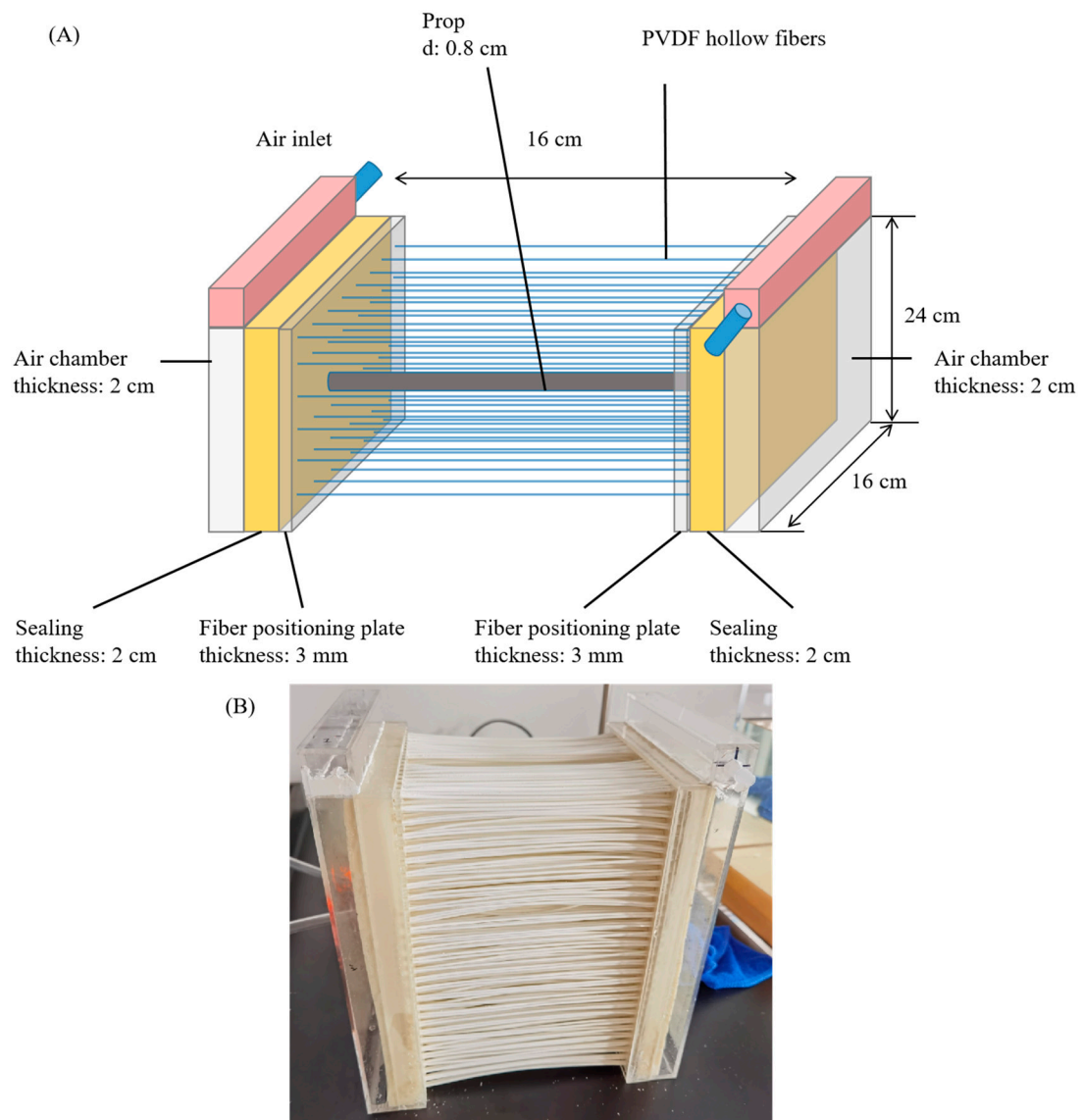
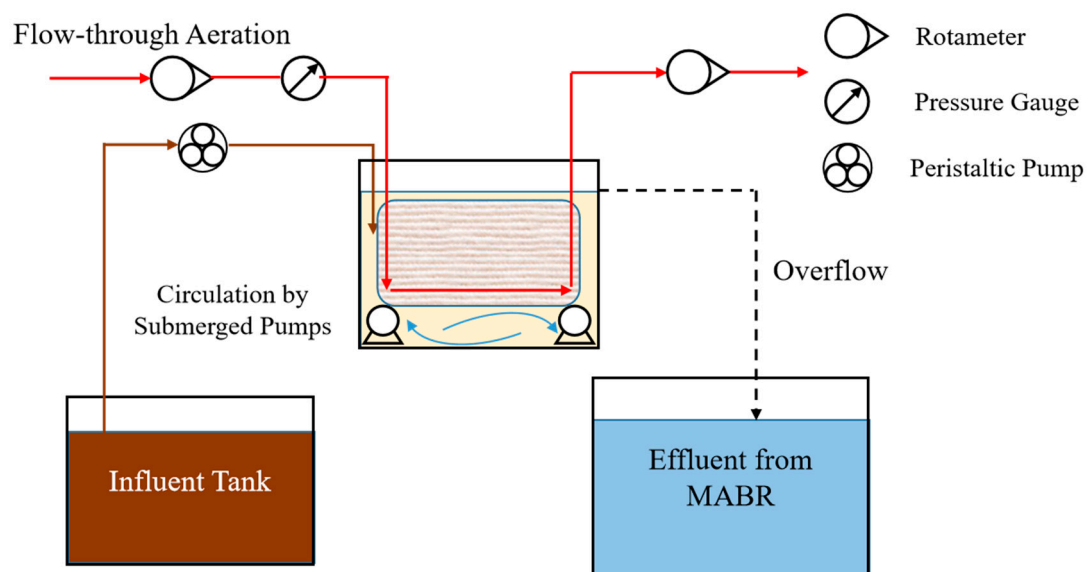


Figure S1. The configuration of the home-made MABR module (A) and its appearance before experiment (B). The module would float in water, so extra weight stacks were attached to the bottom to keep itself immersed during experiment.



Scheme S1. The configuration of the membrane-aerated biofilm reactor (MABR) in this work.

A home-made MABR module was put inside the reactor, and on the reactor bottom was deployed two submerged pumps. The aeration was provided by an air pump, with the inlet and outlet air flow rate measured by rotameters and the aeration pressure measured by a pressure gauge. The influent was fed into the reactor by a peristaltic pump, and the effluent tank was put on a position lower than the reactor so the effluent could overflow by gravity.

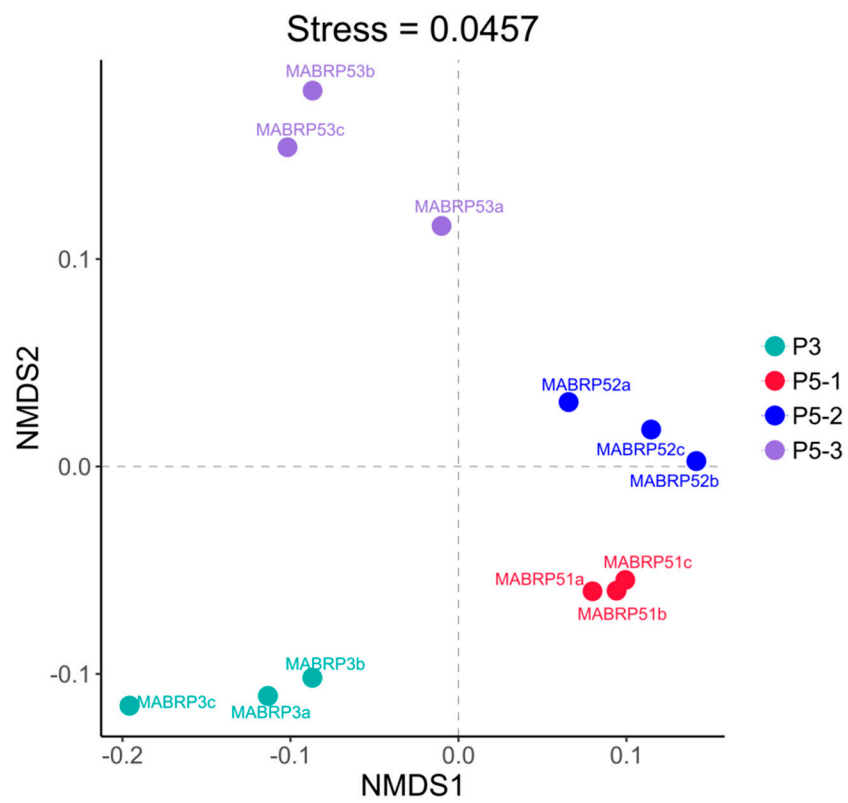


Figure S2. The NMDS plot for biofilm samples taken from P3, P5-1, P5-2 and P5-3.

Tables

Table S1. Composition of nutrient media. The micronutrient components was prepared as a concentrated stock solution (columns on the right) and added to the nutrient media (columns on the left) as a single component.

Nutrient media			
Nutrient media (g/L)		Concentrated micronutrients (g/L)	
(NH ₄) ₂ SO ₄	0.5	CaCl ₂ ·2H ₂ O	0.50
NaHCO ₃	1.0	CoCl ₂ ·6H ₂ O	0.35
KH ₂ PO ₄	1.0	CuSO ₄ ·5H ₂ O	0.15
glucose	0.2	FeCl ₃	0.80
Concentrated micronutrients	3	MgSO ₄ ·7H ₂ O	0.34
		MnCl ₂ ·4H ₂ O	0.50
		Na ₂ MoO ₄ ·2H ₂ O	0.20
		ZnSO ₄ ·5H ₂ O	0.55
		Yeast powder	10

Table S2. The absolute abundance of nitrifying microorganisms detected in biofilm samples taken from BfR at P3, P5-1, P5-2 and P5-3. For each phase 3 parallel samples were taken.

Genus	Denitrifying bacteria					
	P3			P5-1		
	a	b	c	a	b	c
<i>Acidovorax</i>	77086	72324	69168	83287	94127	85466
<i>Alicyclophilus</i>	42012	40101	31153	114808	109042	109862
<i>Thauera</i>	19086	18972	12970	69662	84635	76030
<i>Pseudomonas</i>	42493	55882	43157	50850	46836	40409
<i>Hydrogenophaga</i>	53478	48429	34866	72384	75762	73988
<i>Hyphomicrobium</i>	38581	37327	29754	26745	20831	24685
<i>Methyloversatilis</i>	11895	13136	11042	3195	3025	3192
<i>Burkholderia</i>	24079	24074	16565	20145	19167	19759
<i>Paracoccus</i>	11107	10878	10017	19941	21529	20588
<i>Ralstonia</i>	12516	12812	8555	12791	12670	13228
<i>Cupriavidus</i>	12412	12169	8330	13717	13295	13051
<i>Rubrivivax_gelatinosus</i>	18254	15358	10526	13129	12801	12377
<i>Bradyrhizobium</i>	7600	7693	7438	11121	10165	10127
<i>Thermomonas</i>	4175	3427	3294	1963	2094	2022
<i>Rhizobium</i>	8308	9645	6852	6778	6088	6426
<i>Stenotrophomonas</i>	7270	7034	5291	7021	7114	6709
<i>Azoarcus</i>	4402	4573	2975	8606	8938	8476
<i>Achromobacter</i>	4358	4068	2930	6492	6277	6257
<i>Magnetospirillum</i>	853	1000	747	2279	3006	2829
<i>Bosea</i>	3882	4179	4527	5373	4386	4843
<i>Brevundimonas</i>	9313	11959	9546	3053	2348	2752
<i>Aeromonas</i>	6518	6371	6800	3500	4011	3737
<i>Caulobacter</i>	803	798	813	683	924	775
<i>Rhodopseudomonas</i>	2657	2783	2782	4374	4137	4216
<i>Sulfuritalea</i>	2193	2430	1683	4067	3923	4005
<i>Aquamicrobium</i>	1027	1247	3452	1928	1836	1824
<i>Dechloromonas</i>	1835	1959	1455	2378	2336	2303
<i>Thiobacillus</i>	1642	1924	1187	2316	2228	2245
<i>Arenimonas</i>	1125	1022	973	1653	1695	1588
<i>Alcaligenes</i>	317	351	397	1355	1580	1564
<i>Methylobacillus</i>	109	126	105	198	152	163
<i>Methylothera</i>	15	31	13	30	34	37
<i>Methylophilus</i>	17	17	5	0	15	0

Denitrifying bacteria (continued)

Genus	P5-2			P5-3		
	a	b	c	a	b	c
<i>Acidovorax</i>	83583	76906	80474	84133	82181	81789
<i>Alicyclophilus</i>	68088	69136	70978	76325	68344	70714
<i>Thauera</i>	84771	69926	81040	46103	53858	49494
<i>Pseudomonas</i>	54235	147929	101788	63255	60123	57281
<i>Hydrogenophaga</i>	33668	26142	30343	14839	12485	13135
<i>Hyphomicrobium</i>	24955	24115	25282	37844	44581	41187
<i>Methyloversatilis</i>	3851	4141	3822	66422	76182	71334
<i>Burkholderia</i>	24678	27044	24920	20724	16951	18555
<i>Paracoccus</i>	18667	14614	17373	19652	24815	22641
<i>Ralstonia</i>	16527	17788	17158	18216	16546	17298
<i>Cupriavidus</i>	16352	18160	17297	13660	11715	12405
<i>Rubrivivax_gelatinosus</i>	14146	16817	14417	9174	7563	8267
<i>Bradyrhizobium</i>	13774	11996	13064	13439	16265	15321
<i>Thermomonas</i>	3580	2488	3020	23699	31905	26867
<i>Rhizobium</i>	9214	7973	8999	8430	9765	9191
<i>Stenotrophomonas</i>	7776	8555	8577	10263	10020	10395
<i>Azoarcus</i>	9584	11026	10179	5200	4165	4557
<i>Achromobacter</i>	7784	8066	7839	5482	4313	4749
<i>Magnetospirillum</i>	10511	24202	16576	2245	1505	1120
<i>Bosea</i>	5650	4305	4968	6369	8394	7135
<i>Brevundimonas</i>	2246	3277	2571	3922	3627	3752
<i>Aeromonas</i>	3725	4055	4312	4986	5139	4747
<i>Caulobacter</i>	903	592	754	1011	963	999
<i>Rhodopseudomonas</i>	5683	4939	5470	5926	7003	6456
<i>Sulfuritalea</i>	5810	6099	6255	2527	1451	1968
<i>Aquamicrobium</i>	3322	2401	2764	3618	4300	3820
<i>Dechloromonas</i>	3188	3257	3063	1561	1262	1533
<i>Thiobacillus</i>	3161	3215	3137	1398	1021	1274
<i>Arenimonas</i>	1716	1669	1600	1217	1139	1259
<i>Alcaligenes</i>	561	485	492	514	462	533
<i>Methylobacillus</i>	324	241	282	247	167	258
<i>Methylothera</i>	61	65	69	25	22	23
<i>Methylophilus</i>	22	25	25	32	18	26

Nitrifying bacteria & Anammox bacteria

Genus	P3			P5-1		
	a	b	c	a	b	c
<i>Candidatus Kuenenia</i>	487	497	444	992	614	969
<i>Nitrolancea</i>	0	0	0	7	11	14
<i>Nitrospira</i>	1014	1626	993	175	231	218
<i>Nitrobacter</i>	499	540	1277	833	729	790
<i>Nitrococcus</i>	31	19	9	25	18	26
<i>Nitrosomonas</i>	6473	11924	18220	2078	2247	2365
<i>Nitrosospira</i>	211	223	136	241	173	256
<i>Nitrosovibrio</i>	14	18	7	0	14	0
<i>Nitrosococcus</i>	20	22	23	34	19	38

Nitrifying bacteria & Anammox bacteria (continued)

Genus	P5-2			P5-3		
	a	b	c	a	b	c
<i>Candidatus Kuenenia</i>	4364	4503	5631	1785	991	1503
<i>Nitrolancea</i>	44	47	45	49	49	40
<i>Nitrospira</i>	322	318	307	352	347	365
<i>Nitrobacter</i>	1132	979	1055	1141	1423	1362
<i>Nitrococcus</i>	30	16	26	20	11	25
<i>Nitrosomonas</i>	5421	3423	5780	21925	22648	24953
<i>Nitrosospira</i>	303	298	296	245	215	190
<i>Nitrosovibrio</i>	23	0	29	51	50	29
<i>Nitrosococcus</i>	49	48	56	20	19	40

Table S3. The reads of functional genes involved in nitrification and denitrification pathways detected in biofilm samples taken from MABR at P3, P5-1, P5-2 and P5-3. For each phase 3 parallel samples were taken.

Functional genes	P3			P5-1		
	a	b	c	a	b	c
K10944: <i>amoA</i> EC 1.14.99.39	29.338	36.242	87.618	5.062	10.794	3.503
K10945: <i>amoB</i> EC 1.14.99.39	46.87	81.271	100.87	13.878	13.36	10.237
K10946: <i>amoC</i> EC 1.14.99.39	73.115	102.971	195.376	20.68	6.128	8.436
K10535: <i>hao</i> EC:1.7.2.6	97.199	175.028	176.941	102.547	79.045	96.817
K00370: <i>narG/nxrA</i> EC:1.7.5.1	281.778	249.063	193.132	424.088	381.914	423.432
K00371: <i>narH/ nxrB</i> EC:1.7.5.1	572.008	514.124	489.311	995.614	992.772	1034.4
K00374: <i>narI</i> EC:1.7.5.1	39.525	37.002	46.011	336.843	278.626	274.305
K02567: <i>napA</i> EC:1.9.6.1	57.336	62.404	38.174	74.67	62.371	52.525
K02568: <i>napB</i> EC:1.9.6.1	0	0	0	0	2.499	0
K00368: <i>nirK</i> EC:1.7.2.1	36.755	60.225	79.509	0	1.091	4.929
K15864: <i>nirS</i> EC:1.7.2.1 1.7.99.1	252.312	189.04	154.384	222.286	215.391	244.43
K04561: <i>norB</i> EC:1.7.2.5	52.548	52.775	97.173	52.818	75.277	84.375
K02305: <i>norC</i> EC:1.7.2.5	39.126	36.676	71.515	23.844	35.34	28.686
K00376: <i>nosZ</i> EC:1.7.2.4	215.433	226.45	239.572	446.488	465.739	501.03
K20933: <i>hzs</i> EC:1.7.2.7	36.679	25.817	30.61	85.205	53.526	88.856
K20935: <i>hdh</i> EC:1.7.2.8	20.367	19.241	17.254	53.721	33.338	47.836

Table S3 (continued)

Functional genes	P5-2			P5-3		
	a	b	c	a	b	c
K10944: <i>amoA</i> EC 1.14.99.39	28.148	10.727	14.411	130.996	160.61	174.603
K10945: <i>amoB</i> EC 1.14.99.39	42.259	27.772	33.498	213.424	290.186	256.651
K10946: <i>amoC</i> EC 1.14.99.39	53.63	22.735	56.656	325.693	415.916	419.706
K10535: <i>hao</i> EC:1.7.2.6	525.042	489.434	613.684	540.308	469.583	537.983
K00370: <i>narG/nxrA</i> EC:1.7.5.1	557.151	646.787	645.135	305.2	228.207	247.038
K00371: <i>narH/ nxrB</i> EC:1.7.5.1	1109.657	1040.166	1044.685	624.382	609.631	615.782
K00374: <i>narI</i> EC:1.7.5.1	85.903	60.215	27.925	36.008	72.294	40.599
K02567: <i>napA</i> EC:1.9.6.1	98.271	98.69	111.471	58.203	39.327	11.45
K02568: <i>napB</i> EC:1.9.6.1	7.728	12.935	13.469	0	14.029	14.306
K00368: <i>nirK</i> EC:1.7.2.1	15.873	3.975	0	61.14	77.765	81.362
K15864: <i>nirS</i> EC:1.7.2.1 1.7.99.1	155.019	162.382	165.636	140.349	139.751	115.61
K04561: <i>norB</i> EC:1.7.2.5	39.349	26.071	41.949	84.01	116.752	116.545
K02305: <i>norC</i> EC:1.7.2.5	42.256	26.555	37.243	107.174	131.076	172.669
K00376: <i>nosZ</i> EC:1.7.2.4	522.381	532.262	592.821	274.124	182.876	211.353
K20933: <i>hzs</i> EC:1.7.2.7	315.535	353.28	380.022	115.378	67.05	81.651
K20935: <i>hdh</i> EC:1.7.2.8	205.113	214.087	244.823	74.538	40.35	58.27