Supplementary material

Moving bed biofilm reactor for blackwater treatment: Insights into pollutant removal, microbial communities, and water quality prediction through machine learning

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Text S1 Simulated wastewater characteristics and operational procedures

The COD of the wastewater was contributed by starch, glucose, sodium acetate and sodium propionate in a ratio of 1:4:2:2. The other components included: 458.6 mg/L of NH_4Cl, 52.8 mg/L of KH_2PO_4, 38.2 mg/L of KCl, 76.1 mg/L of NaCl, 21.7 mg/L of KNO_3, 9.9 mg/L of $NaNO_2$, 4.3 mg/L of $FeCl_3 \cdot 6H_2O$, 0.37 mg/L of $ZnSO_4$, 0.11 mg/L of Na_2MoO_4, 0.14 mg/L of $MnCl_2$, 0.08 mg/L of $CuSO_4$, 0.44 mg/L of $CaCl_2$, 0.74 mg/L of $MgSO_4$, and 0.1 mg/L of EDTA. All reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd., China.

The inoculum employed in the reactor was obtained from Wusong Wastewater Treatment Plant, Shanghai, China. The mixed liquid suspended solids (MLSS) of the inoculum sludge was 11.96

g/L and the mixed liquid volatile suspended solids (MLVSS) was 8.49 g/L. Bio-carrier used was made of polyurethane (PU) with a quadrate shape (30 × 30 × 30 mm) and a specific surface area of 4000 m^2/m^3. The MBBR was operated with 30% filling rate (volume ratio of bio-carriers to reactor).

The setup was operated for 82 days according to six experimental phases. The influent features of the wastewater and the operational parameters during the six phases were summarized in Table S1.

Text S2 Experimental process for blackwater and greywater treatment

The conventional suspended sludge system has demonstrated efficacy in reducing COD and total suspended solids, as well as facilitating nitrification. Nevertheless, the efficiency of wastewater treatment plants in many northern regions of China remains suboptimal, particularly under low temperature conditions, posing persistent challenges. This section explores the operational adaptability of the reactor to low temperatures. Given the limited adoption of blackwater and greywater segregation in rural areas of China, a mixture of blackwater and greywater is utilized to investigate the impact of diverse domestic water sources, including bathwater, kitchen wastewater, and other miscellaneous sources.

In this experiment, simulated blackwater and the mix of blackwater and greywater were used as the influent. Sodium dodecylbenzene sulfonate was applied to adjust the concentration of Linear Alklybezene Sulfonates (LAS). The influent water quality was shown in Table S2. The simulated wastewater had some bubbles and was turbid. The HRT of the reactor was set as 25.5 h, with a reflx ratio of 110%. Intermittent aeration (5 h/1 h) was employed in O1 and O2 zones, and polyurethane porous gel carriers (PPC) were added into the anoxic zone A1 and A2. The specific surface area was over 4000 m^2/m^3, with a porosity of 98%, and a filling ratio of 30%. The indoor temperature was controlled by an air conditioner or a refrigerator, and the water temperature was measured using an alcohol thermometer.

Text S3 Dehydrogenase activity analysis method

Pipette 1, 2, 3, 4, 5, and 6 mL of a 1 mg/mL 2,3,5-triphenyl tetrazolium chloride (TTC standard solution) into separate 50 mL brown volumetric flasks. Add deionized water to each flask to dilute to 50 mL and mix well to obtain TTC series solutions with concentrations of 20, 40, 60, 80, 100, and 120 μg/mL, respectively. Next, take seven centrifuge tubes. To one tube, add 1 mL of deionized water; to the other six tubes, add 1 mL of the corresponding TTC series solution. In each tube, sequentially add 2 mL of 0.1% glucose solution, 2 mL of Tris-HCl buffer solution, and 1 mL of freshly prepared 10% Na_2S solution. Shake thoroughly and allow the solution to develop color for 10 min. Then add 5 mL of toluene, shake well, and once clear phase separation is observed, centrifuge at 5000 r/min for 10 min. Finally, take the upper organic layer and measure its absorbance at 492 nm, then plot the concentration–absorbance standard curve.

Text S4 Ammonia absorption rate and nitrate absorption rate testing procedure

Ten carriers were taken from each of the O1 and O2 tanks, as these carriers might contain solutions with ions such as ammonia nitrogen and nitrate nitrogen. The washed-down biomass was collected, and the carriers were rinsed with deionized water. Both the carriers and the collected biomass were placed into the matrix. Additionally, 500 mL of liquid from each of the O1 and O2 tanks was collected to separate the suspended biomass. The composition of NUR and AUR experimental matrices is listed in Table S3. A sufficient amount of glucose was added to the NUR matrix to ensure that the carbon source was not a limiting factor. To eliminate the influence of nitrifying bacteria, allylthiourea (ATU) was added as a nitrification inhibitor to the synthetic NUR matrix. The addition of K^+, Na^+, and other trace elements met the growth requirements of microorganisms. For denitrification rate tests, the flask was flushed with nitrogen for 10 min until the dissolved oxygen remained at zero, then sealed. AUR experiments were conducted in a 5 L plastic graduated cylinder, aerated, and dissolved oxygen was maintained at 2-3 mg/L, while NUR experiments were conducted in a 1 L sealed flask, with continuous stirring (Fig. S1). By periodically sampling and determining the concentrations of NH_3-N, NO_3^--N and NO_2^--N, AUR and NUR were obtained through data fitting, divided by the corresponding initial attached growth biomass (AGB) and suspended growth sludge (SGS) quantities, the nitrification and denitrification capabilities per unit biomass could be calculated.

Text S5 DNA extraction and analysis procedures

The microbial samples were collected from the reactor and preserved at −80℃ for DNA extraction. DNA extraction for the microbial samples was conducted using an AxyPrep DNA Gel Extraction Kit (USA), according to the instruction of manufacturer. The V3-V4 regions of the 16S rRNA gene were amplified using the prime pair 338F (5′-ACTCCGGGAGGCAG-3′)/806R (5′-GGactAchVGGTWTCTAAT-3′) by an ABI GeneAmp®9700 PCR Thermal Cycler. Concentration and purity of the extracted DNA samples were determined using Quantus™ Fluorometer (Promega, USA) and NanoDrop 2000 UV-vis (Thermo Scientific, USA), respectively. The data were analyzed through the Major Cloud Platform of the online free website.

Fig. S1 Images for AUR and NUR batch experiments.

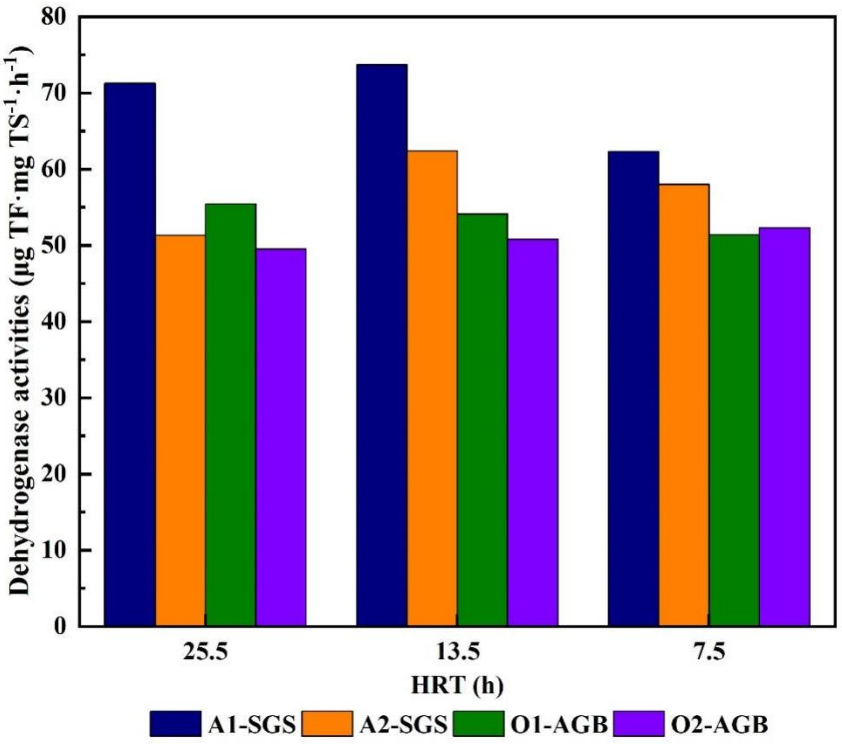

Fig. S2 Dehydrogenase activities of SGS and AGB under different HRTs.

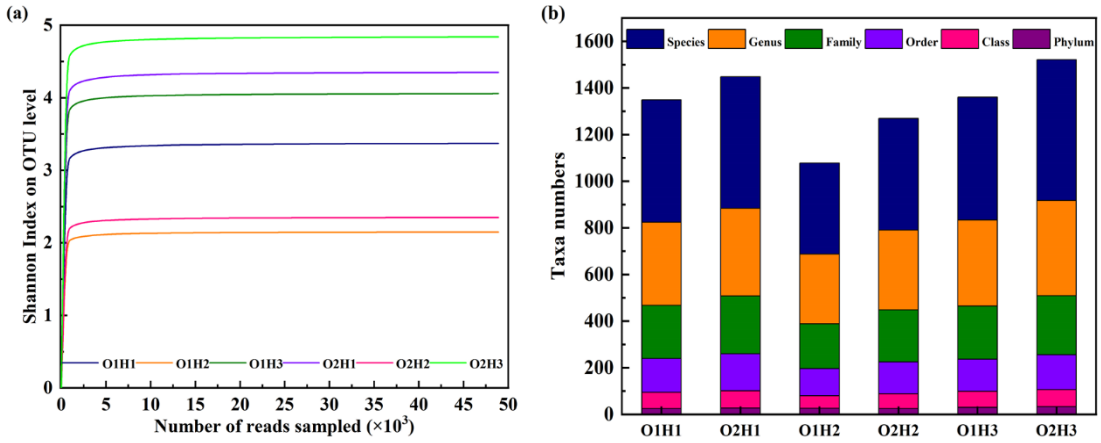

Fig. S3 Sequencing information of biofilm samples: (a) Rarefaction curve, (b) Taxa numbers at different taxonomic levels.

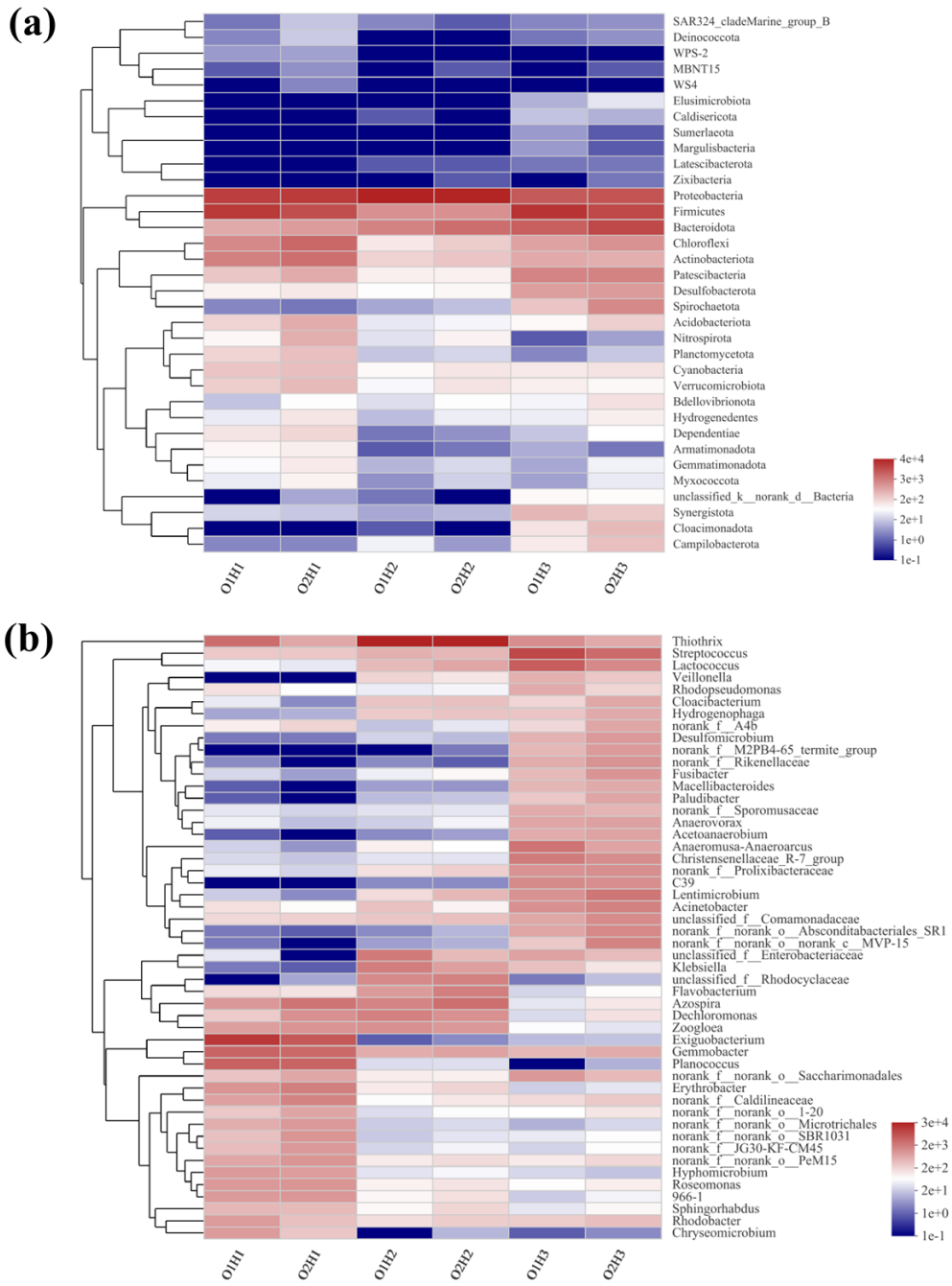

Fig. S4 Heatmap of microbial community at (a) phylum and (b) genus level.

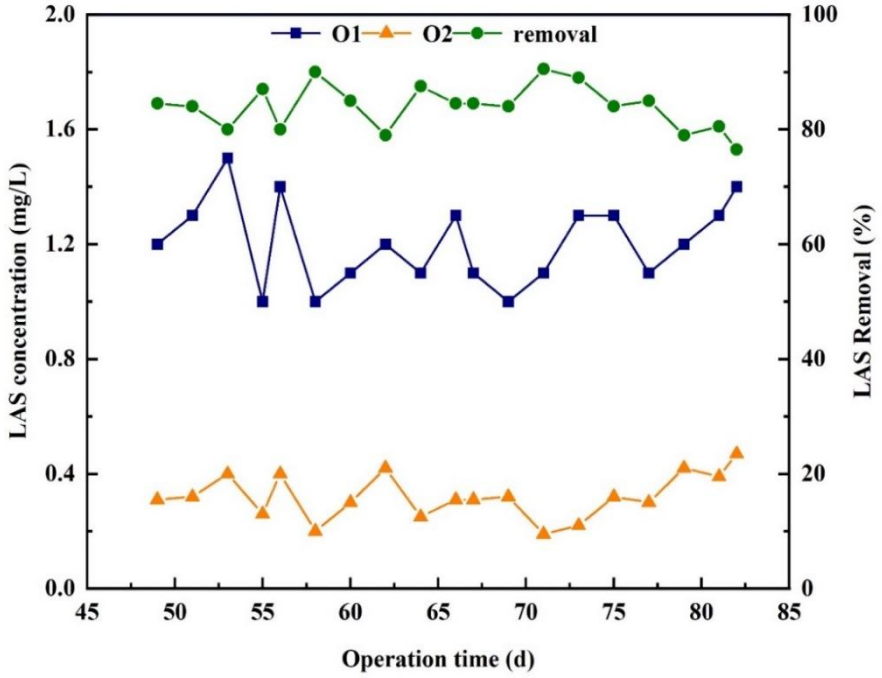

Fig. S5 LAS removal by MBBR.

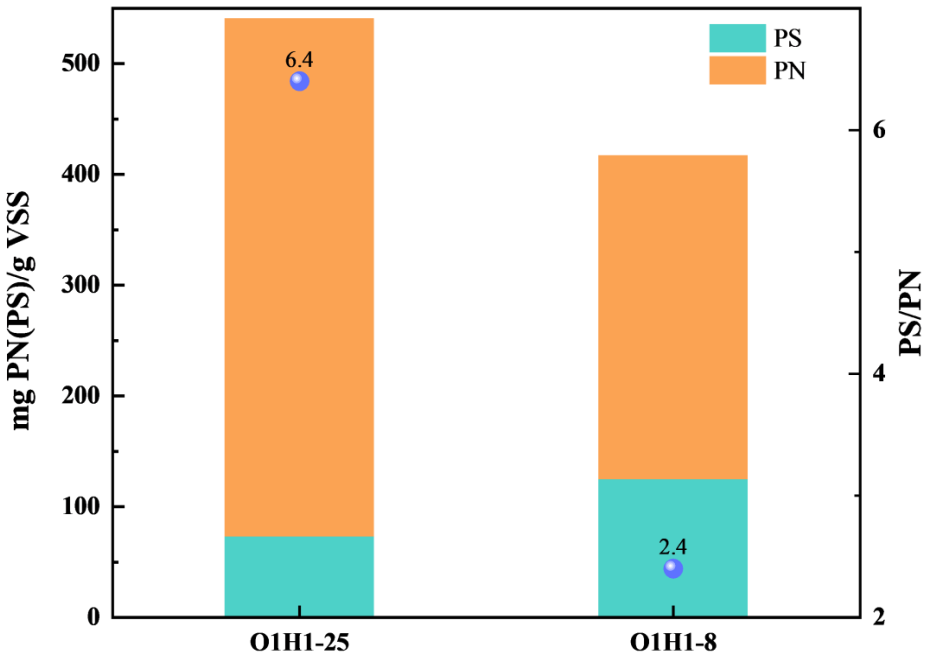

Fig. S6 The biofilm PN and PS concentrations of O1H1-25 and O1H1-8.

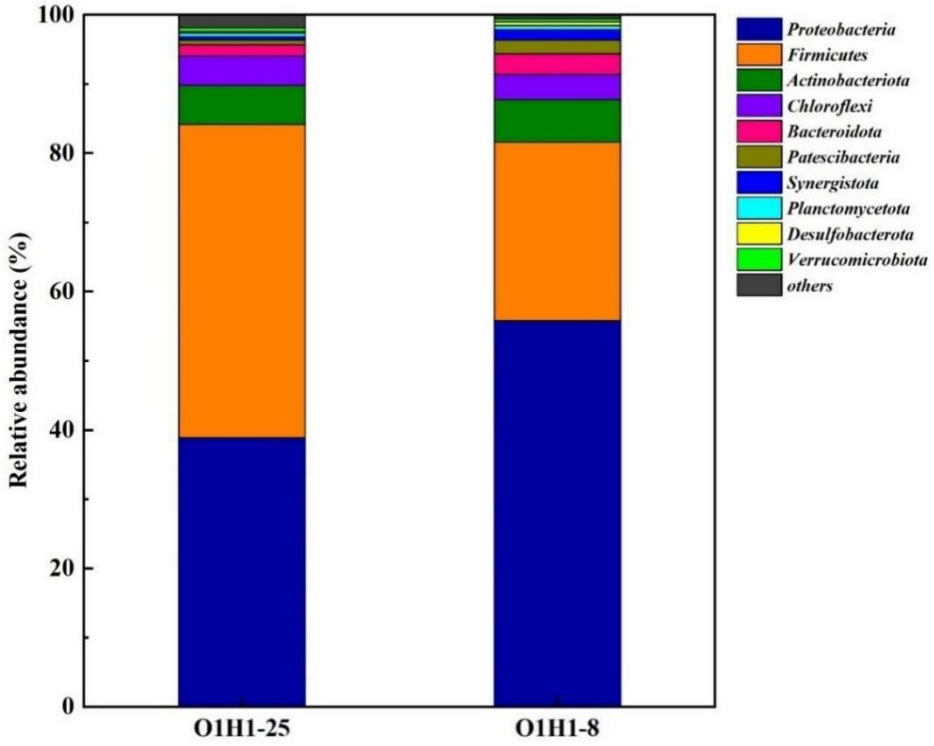

Fig. S7 Relative abundance distribution of the microbial community at the phylum level in samples O1H1-25 and O1H1-8.

Table S1 Influent quality and operational parameters for the wastewater treatment process.

Experimental phase	**COD (mg/L)**	**NH_3-N (mg/L)**	**TN (mg/L)**	**TP (mg/L)**	**HRT (h)**	**Temperature (℃)**	**Duration (d)**
1	1500	120	130	12	25.5	25	10
2	1500	120	130	12	13.5	25	10
3	1500	120	130	12	7.5	25	10
4	1500	120	140	12	25.5	15	18
5	800	85	100	6	25.5	13	22
6	800	85	100	6	25.5	8	12

Table S2 Influent water quality (mg/L).

Influent	COD	NH_3-N	NO_3^--N	NO_2^--N	TN	TP	LAS
BW	1500	120	3	2	140	12	0
BW + GW	800	85	3	2	100	6	2

Table S3 NUR and AUR experimental matrix (mg/L).

Matrix	V(L)	Glucose	NH_3-N	NO_3^--N	ATU	K^+	Na^+	Other trace elements
NUR	0.75	1000	0	45	2	2	3	0.05
AUR	1.5	0	60	0	0	2	3	0.05

Table S4 Sequencing information, OTU and alpha diversity of biofilm samples.

Sample	Sequences	OTU	Shannon	Simpson	Ace	Chao	Coverage
O1H1	53353	701	3.37	0.127	849.73	820.90	0.997
O1H2	49455	463	2.15	0.371	679.42	655.89	0.997
O1H3	69002	689	4.06	0.063	788.40	792.27	0.996
O2H1	55308	774	4.35	0.040	860.93	869.82	0.997
O2H2	73564	544	2.35	0.349	715.70	701.25	0.996
O2H3	74781	785	4.84	0.017	899.20	902.63	0.997

Table S5 Comparison of biofilms sequencing information of O1H1-25 and O1H1-8.

Sample ID	Sequences	OTU	Shannon	Simpson	Ace	Chao	Coverage
O1H1-25	53353	701	3.371	0.127	849.725	820.897	0.997
O1H1-8	59873	477	2.149	0.371	679.424	655.887	0.997

Table S6 Fitting indicators of ML model under training stage.

Chamber	NH_3-N			TN			TP		
	R^2	MAE	RMSE	R^2	MAE	RMSE	R^2	MAE	RMSE
A1	0.953	1.79	2.336	0.960	1.680	2.246	0.946	0.231	0.307
O1	0.983	1.608	2.265	0.970	1.733	2.508	0.954	0.306	0.410
A2	0.984	1.631	2.235	0.975	1.641	2.322	0.994	0.191	0.263
O2	0.983	1.423	1.884	0.961	1.669	2.301	0.967	0.296	0.409

Table S7 Fitting indicators of ML model under test stage.

Chamber	NH_3-N			TN			TP		
	R^2	MAE	RMSE	R^2	MAE	RMSE	R^2	MAE	RMSE
A1	0.520	5.598	7.322	0.523	5.866	7.612	0.655	0.554	0.736
O1	0.678	6.512	9.530	0.574	6.475	9.190	0.789	0.897	1.209
A2	0.613	7.640	10.487	0.543	6.514	9.489	0.957	0.627	0.827
O2	0.635	5.346	8.404	0.490	5.665	7.894	0.927	0.625	0.915

Table S8 Simulation of water quality for different influent loads and operating conditions.

Stage	Operation days	Influent NH_3-N (mg/L)	Influent TN (mg/L)	Influent TP (mg/L)	Temperature (℃)	HRT (h)
1	10	240	260	20	25	25
2	10	120	130	12	5	25
3	10	120	130	12	25	4