Supplementary Information

Food wastewater treatment by hybrid biofilm reactor: Nutrients removal performance and functional microorganisms on filler biofilm and suspended sludge

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Contents

Methods S1 The determination of biofilm mass.

Take 30 fillers from the column and dry them in a 105°C oven for more than 5 hours. After the fillers have cooled to room temperature, weigh them and record them as W_1. Then, place the filler in a 1 mol/L 70°C NaOH solution and continuously stir for 1 hour. The hot alkaline solution can break the adhesive between the microorganisms on the biofilm and the filler carrier. After removing the filler, rinse it thoroughly and place it in an ultrasonic cracking tank for 30 minutes (40Hz). The stripped clean filler is then placed in a 105°C oven and dried for 5 hours. After cooling to room temperature, it is weighed and recorded as W_2. The amount of biofilm (m) on the filler can be calculated by:

$$m = \frac{M_1 - M_2}{M_2} \times 10^3 \quad \text{(mg/g)}$$
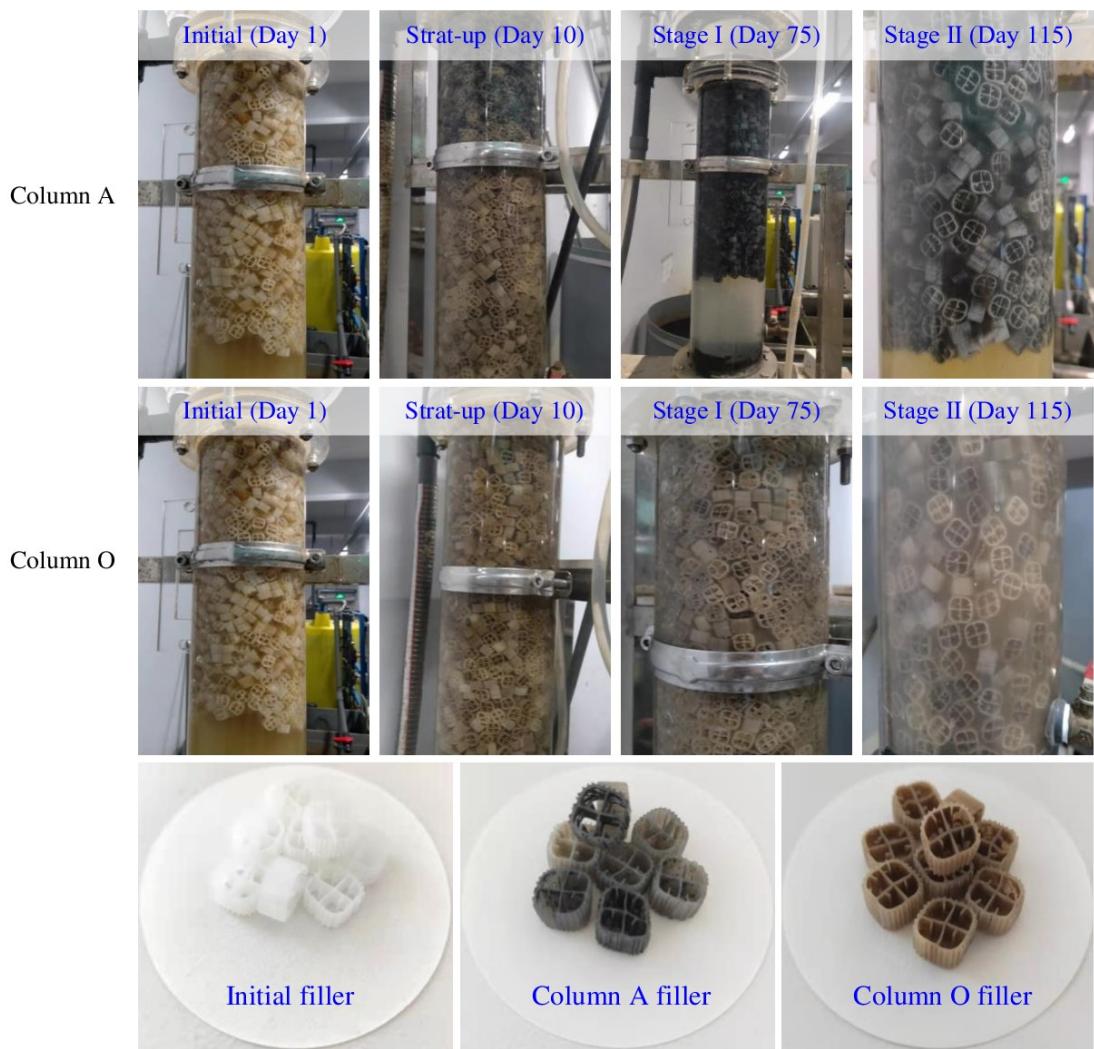
Fig. S1 Filler biofilm status at different experimental stages.
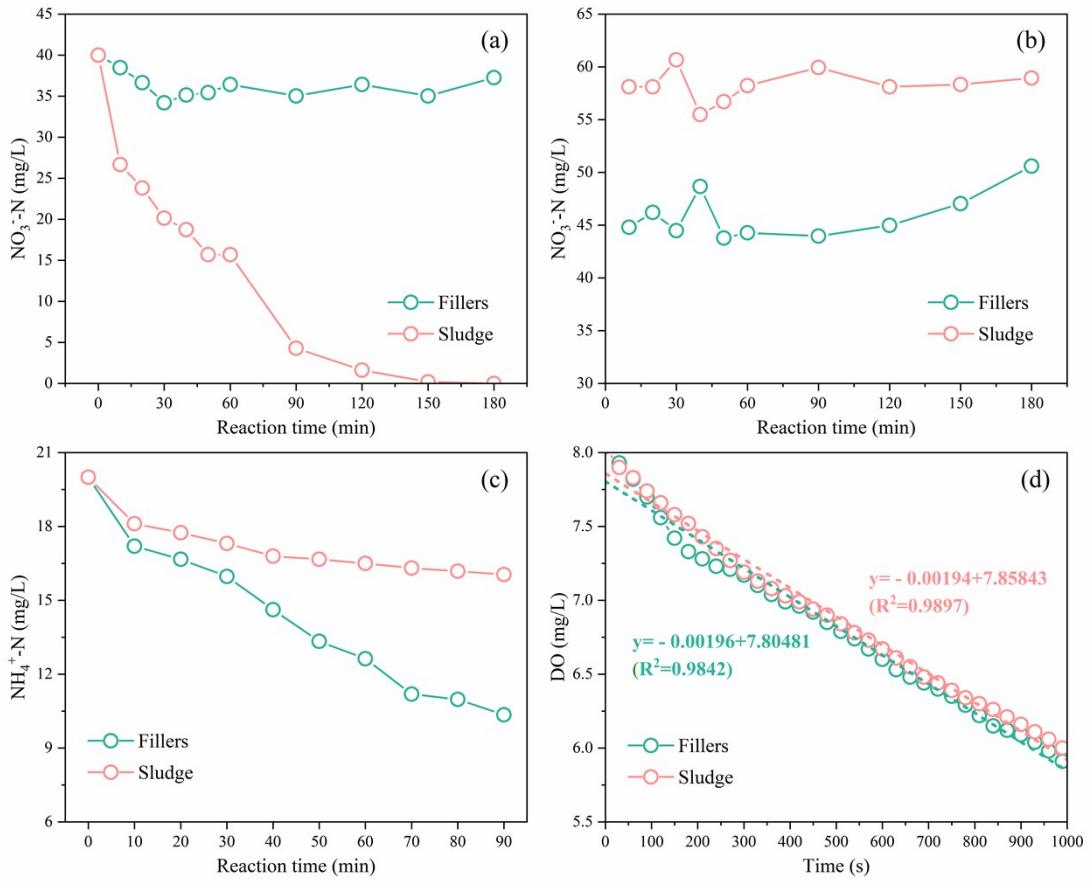
Fig. S2 (a) Denitrification activity of fillers and sludge in column A (a), and column O (b); Nitrification activity of fillers and sludge in column O (c), and oxygen consumption rate curve of fillers and sludge in column O (d).
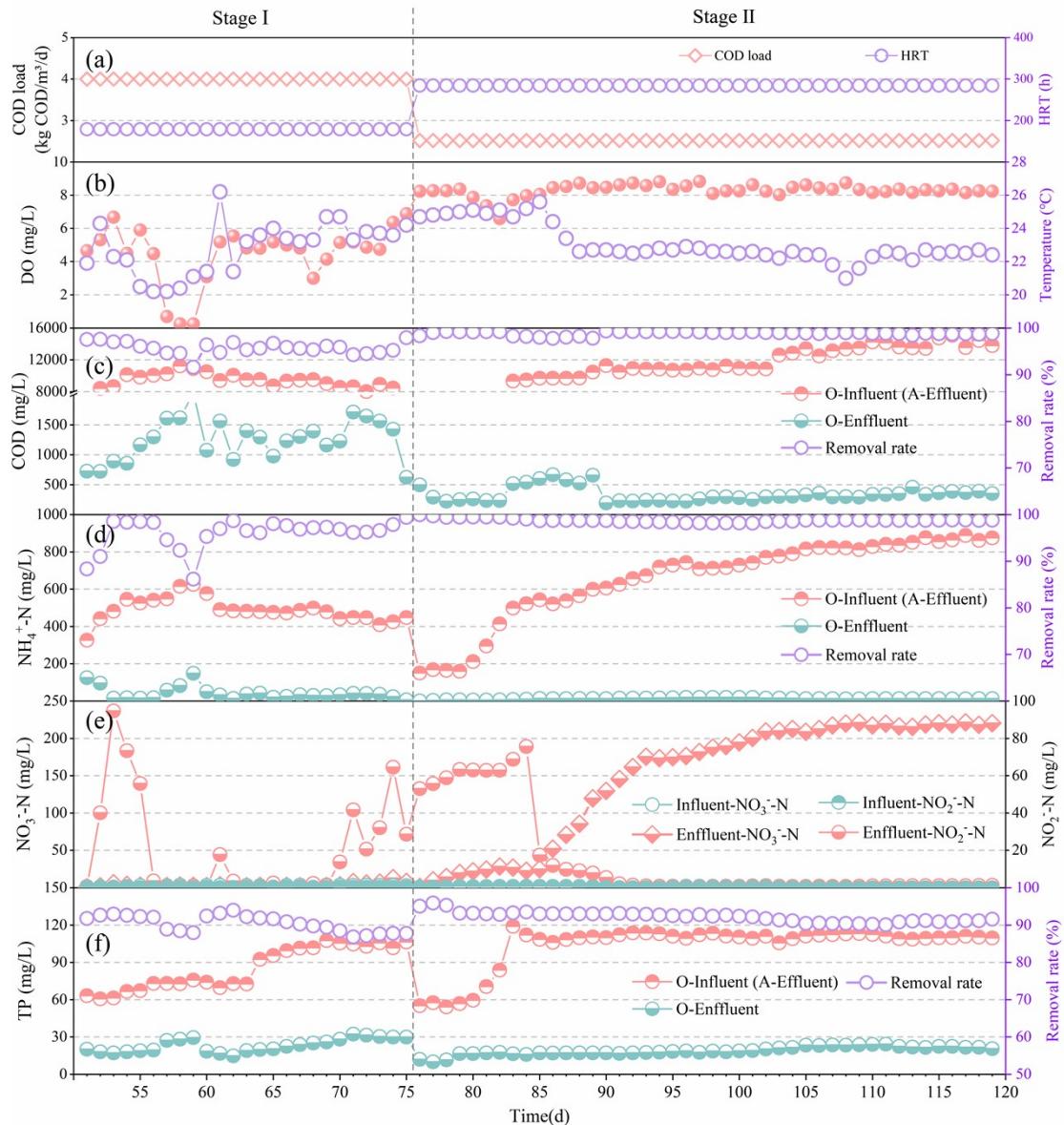
Fig. S3 Operation status of the column O: (a) COD load and HRT at different experimental stages, and (b) temporal trends in DO and temperature; nutrient-removal profiles of (c) COD, (d) NH_4^+-N, (e) NO_3^--N and NO_2^--N, and (f) TP.

(a)

FWW → Degreasing → UASB → HBR → Effluent

UASB → Sludge Treatment ← HBR
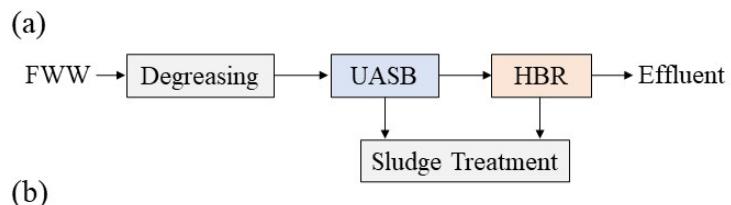
(b)

Unit	Designed influent COD concentration (mg/L)	Designed effluent COD concentration (mg/L)	COD removal rate (%)	HRT (h)
UASB	30000	4500	85%	61.2
HBR	4500	< 500	97%	57.14
			Total	118.34

Fig. S4 (a) The flowchart of the UASB-HBR process, and (b) the envisioned performance of the UASB+HBR process for treating FWW.

Table S1 The characteristics of the FWW (mg/L).

	Chemical oxygen demand (COD)	Ammonia nitrogen (NH_4^+-N)	Total nitrogen (TN)	Total phosphorus (TP)
FWW	29860 ± 276	1072.3 ± 14.6	1245.7 ± 134.7	245.1 ± 15.3
Effluent standards	500	/	/	/

/ indicates that there is no limit on the effluent concentration of this parameter.

Table S2 Alpha diversity of total bacterial 16S rRNA gene in the HBR system.

Sample	ace	chao	coverage	shannon	simpson	sobs
F-A-P1-1	1258.45	1204.18	0.9921	4.7824	0.03150	1031
F-A-P1-2	1296.39	1266.96	0.9915	4.7791	0.0323	1043
F-A-P1-3	1275.64	1210.78	0.9919	4.7314	0.0350	1034
S-A-P1-1	1292.99	1222.60	0.9912	4.6272	0.0279	1027
S-A-P1-2	1304.38	1263.10	0.9908	4.4811	0.0329	1003
S-A-P1-3	1239.46	1170.54	0.9917	4.5200	0.0314	988
F-O-P1-1	1273.13	1251.72	0.9915	4.6601	0.0353	1015
F-O-P1-2	1248.40	1212.40	0.9916	4.6326	0.0352	984
F-O-P1-3	1232.04	1176.88	0.9923	4.6037	0.0380	1027
S-O-P1-1	1059.02	1020.78	0.9924	4.3212	0.0332	818
S-O-P1-2	1106.82	1099.16	0.9917	4.2499	0.0369	826
S-O-P1-3	1042.41	1004.57	0.9925	4.2602	0.0362	795
F-A-P2-1	1397.46	1378.04	0.9913	5.3907	0.0105	1143
F-A-P2-2	1391.91	1392.73	0.9913	5.3741	0.0112	1156
F-A-P2-3	1501.06	1448.06	0.9905	5.4242	0.0103	1230
S-A-P2-1	1427.55	1381.97	0.9896	4.6741	0.0284	1066
S-A-P2-2	1329.92	1270.23	0.9908	4.6543	0.0303	1038
S-A-P2-3	1382.59	1377.50	0.9897	4.6136	0.0313	1018
F-O-P2-1	1152.67	1133.62	0.9930	4.7146	0.0282	966
F-O-P2-2	1225.84	1188.61	0.9923	4.7523	0.0274	1015
F-O-P2-3	1177.82	1143.89	0.99263	4.6969	0.0285	968
S-O-P2-1	1108.70	1041.41	0.9922	4.1376	0.0595	859
S-O-P2-2	1101.54	1032.87	0.9924	4.1427	0.0558	864
S-O-P2-3	1106.54	1077.58	0.9920	4.1607	0.0533	834

Table S3 Carbohydrate metabolism pathways and the abundance of metabolism genes in HBR system.

Metabolic pathways	Pathway modules	Metabolism	F-A-P1	S-A-P1	F-O-P1	S-O-P1	F-A-P2	S-A-P2	F-O-P2	S-O-P2
Glycolysis / Gluconeogenesis	M00001	Glycolysis (Embden-Meyerhof pathway)	349538.88	377707.42	389636.68	373014.56	375335.80	363486.95	386219.73	381399.29
	M00002	Glycolysis, core module involving three-carbon compounds	218688.38	228672.85	248098.27	225851.20	229019.36	223835.29	244402.67	236925.59
	M00003	Gluconeogenesis	262729.46	284966.51	299057.73	283084.10	283364.67	269552.88	291470.87	290725.26
	M00307	Pyruvate oxidation	135156.93	201707.03	179433.83	186414.51	143693.26	198479.40	189168.63	202933.44
Citrate cycle (TCA cycle)	M00003	Gluconeogenesis	262729.46	284966.51	299057.73	283084.10	283364.67	269552.88	291470.87	290725.26
	M00307	Pyruvate oxidation	135156.93	201707.03	179433.83	186414.51	143693.26	198479.40	189168.63	202933.44
	M00009	Citrate cycle (TCA cycle, Krebs cycle)	393191.55	509322.09	532551.55	507395.63	403844.84	498792.99	511538.19	475843.50
	M00010	Citrate cycle, first carbon oxidation	75134.57	95968.20	120521.87	96092.18	79297.93	89876.86	112150.11	89297.06
	M00011	Citrate cycle, second carbon oxidation	318056.98	413353.90	412029.68	411303.45	324546.91	408916.13	399388.08	386546.44
	M00740	Methylaspartate cycle	89544.56	141280.67	161559.36	143453.10	99123.77	121620.90	153598.71	127597.22
Pentose phosphate pathway	M00004	Pentose phosphate pathway (Pentose phosphate cycle)	186192.39	242576.59	242268.15	255248.69	203432.99	226972.66	235845.29	251905.83
	M00006	Pentose phosphate pathway, oxidative phase	28176.57	56742.93	74497.05	65869.41	34020.25	49343.03	70459.75	73368.27
	M00007	Pentose phosphate pathway, non-oxidative phase	131359.22	152237.95	139478.01	155147.15	140882.31	145248.99	136732.12	142908.71
	M00005	PRPP biosynthesis	31398.28	40126.08	31689.46	37858.84	29836.04	41338.16	32106.04	41165.35
	M00008	Entner-Doudoroff pathway	33709.20	65040.97	74225.54	76029.25	41403.14	55875.71	67295.49	77863.08
	M00308	Semi-phosphorylative Entner-Doudoroff pathway	91853.47	110784.45	96892.40	113585.64	94673.25	103823.03	92181.08	115808.15
	M00633	Semi-phosphorylative Entner-Doudoroff pathway	391.27	3361.66	2333.73	3624.82	1040.78	3629.25	2890.11	5943.21
	M00309	Non-phosphorylative Entner-	9524.74	8060.90	3280.38	3888.60	19361.04	8921.48	5557.54	3744.82

		Doudoroff pathway								
Pentose and glucuronate interconversions	M00014	Glucuronate pathway (uronate pathway)	49078.00	54133.68	55801.94	54858.36	60824.80	51922.07	66466.53	51740.53
	M00631	D-Galacturonate degradation (bacteria)	38295.74	59954.54	42267.09	62786.50	40085.07	54705.94	38013.16	67628.84
	M00061	D-Glucuronate degradation	38681.70	54840.98	46740.44	59467.77	42657.54	52655.59	42247.90	64800.00
	M00081	Pectin degradation	301.06	7045.05	548.89	7627.00	235.48	7472.07	777.20	12197.32
Galactose metabolism	M00633	Semi-phosphorylative Entner-Doudoroff pathway	391.27	3361.66	2333.73	3624.82	1040.78	3629.25	2890.11	5943.21
	M00309	Non-phosphorylative Entner-Doudoroff pathway	9524.74	8060.90	3280.38	3888.60	19361.04	8921.48	5557.54	3744.82
	M00632	Galactose degradation, Leloir pathway	71011.32	90050.25	94366.66	91429.20	75387.16	80971.07	98611.59	104408.32
	M00552	D-galactonate degradation, De Ley-Doudoroff pathway	69820.12	84585.74	85542.24	88333.82	71899.38	78354.55	79803.61	84491.81
	M00554	Nucleotide sugar biosynthesis	12976.49	20141.84	17226.70	17455.59	14283.33	17465.42	20643.27	22978.91
Starch and sucrose metabolism	M00854	Glycogen biosynthesis	94089.58	92750.91	101323.62	84151.55	96857.89	92456.44	91844.32	79766.39
	M00855	Glycogen degradation	75946.27	74795.46	77458.83	68210.80	78298.89	83693.74	72957.11	70135.53
	M00565	Trehalose biosynthesis	73518.46	73179.98	84292.27	63303.86	77435.30	73643.88	75302.97	61172.66
Amino sugar and nucleotide sugar metabolism	M00549	Nucleotide sugar biosynthesis	76572.65	89317.37	100284.57	88815.98	87501.53	84966.79	97740.76	83322.29
	M00554	Nucleotide sugar biosynthesis	12976.49	20141.84	17226.70	17455.59	14283.33	17465.42	20643.27	22978.91
	M00892	UDP-N-acetyl-D-glucosamine biosynthesis, eukaryotes	82934.50	101926.15	101375.89	103223.11	92113.35	95639.30	102270.88	95906.89
	M00909	UDP-N-acetyl-D-glucosamine biosynthesis, prokaryotes	127319.44	142802.78	153744.15	143345.26	143608.92	137756.76	155349.69	139742.74
	M00761	Undecaprenylphosphate alpha-L-Ara4N biosynthesis	2076.49	843.05	445.97	665.89	3717.11	1568.21	268.00	321.09
Pyruvate metabolism	M00307	Pyruvate oxidation	135156.93	201707.03	179433.83	186414.51	143693.26	198479.40	189168.63	202933.44
	M00172	C4-dicarboxylic acid cycle, NADP -	37435.23	58965.07	55498.53	62513.32	38649.34	55138.02	50850.55	55678.12

		malic enzyme type								
	M00579	Phosphate acetyltransferase-acetate kinase pathway	51866.09	30402.28	33718.24	24651.95	44088.12	37765.24	31357.27	29596.56
Glyoxylate and dicarboxylate metabolism	M00012	Glyoxylate cycle	73209.65	109697.51	154449.46	118096.21	82545.29	99066.58	143279.25	103859.33
	M00373	Ethylmalonyl pathway	103376.19	207084.68	265153.45	225349.28	112256.88	191454.59	244343.36	211722.31
	M00740	Methylaspartate cycle	89544.56	141280.67	161559.36	143453.10	99123.77	121620.90	153598.71	127597.22
	M00532	Photorespiration	132111.01	205540.32	215070.23	208272.58	139232.64	198386.49	204490.03	205648.88
	M00741	Propanoyl-CoA metabolism	52965.76	102265.22	131786.94	116481.89	61202.39	93359.68	123269.60	96848.84
Propanoate metabolism	M00013	Malonate semialdehyde pathway	21015.56	20874.58	51165.54	20846.34	22933.75	18616.71	48126.28	22583.44
	M00741	Propanoyl-CoA metabolism	52965.76	102265.22	131786.94	116481.89	61202.39	93359.68	123269.60	96848.84

Table S4 Abundance of nitrogen metabolism genes in HBR system.

Metabolic pathways	Enzyme	KO	Functional genes	F-A-P1	S-A-P1	F-O-P1	S-O-P1	F-A-P2	S-A-P2	F-O-P2	S-O-P2
Complete nitrification, comammox	1.14.99.39	K10944	*pmoA-amoA*	15.33	3.50	33.00	2.67	26.17	1.50	34.17	2.67
	1.7.2.6	K10535	*hao*	2.83	2.34	24.83	1.34	6.83	0.67	23.17	0.50
	1.7.99.-	K00370	*narG, narZ, nxrA*	1533.98	2439.26	7527.90	1460.14	840.12	3245.05	6082.61	2223.38
Nitrification	1.14.99.39	K10944	*pmoA-amoA*	15.33	3.50	33.00	2.67	26.17	1.50	34.17	2.67
	1.7.2.6	K10535	*hao*	2.83	2.34	24.83	1.34	6.83	0.67	23.17	0.50
Denitrification	1.7.99.-	K00370	*narG, narZ, nxrA*	1533.98	2439.26	7527.90	1460.14	840.12	3245.05	6082.61	2223.38
	1.9.6.1	K02567	*napA*	2438.05	2404.77	913.66	1364.20	1525.58	3323.04	1166.89	2165.52
	1.7.2.1	K00368	*nirK*	549.23	4210.61	3317.02	4355.83	890.57	3880.90	3580.00	6382.16
	1.7.2.5	K04561	*norB*	1959.23	3308.31	2883.02	2904.00	2791.27	4452.68	2512.97	4059.25
	1.7.2.4	K00376	*nosZ*	1941.93	8100.53	2554.88	7047.47	1270.46	7871.41	2977.33	9404.16
Assimilatory nitrate reduction	1.7.99.-	K00370	*narG, narZ, nxrA*	1533.98	2439.26	7527.90	1460.14	840.12	3245.05	6082.61	2223.38
	1.7.2.2	K00367	*narB*	748.56	6097.45	1443.44	5798.89	551.33	4722.78	645.45	6800.00
	NR	K10534	*NR*	0.01	1.01	0.01	0.01	0.01	0.01	0.01	0.01
	1.7.7.1	K00366	*nirA*	925.10	292.83	512.45	396.00	707.93	186.31	765.46	501.15
	1.7.1.4	K17877	*NIT-6*	-	-	-	-	-	-	-	-
Dissimilatory nitrate reduction	1.7.99.-	K00370	*narG, narZ, nxrA*	1533.98	2439.26	7527.90	1460.14	840.12	3245.05	6082.61	2223.38
	1.7.5.1	K00370	*narG, narZ, nxrA*	1533.98	2439.26	7527.90	1460.14	840.12	3245.05	6082.61	2223.38
	1.9.6.1	K02567	*napA*	2438.05	2404.77	913.66	1364.20	1525.58	3323.04	1166.89	2165.52
	1.7.1.15	K00362	*nirB*	3503.63	10979.94	20353.87	10430.37	3659.93	10847.16	17574.96	12588.49
	1.7.2.2	K00367	*narB*	748.56	6097.45	1443.44	5798.89	551.33	4722.78	645.45	6800.00
Anammox	1.7.2.1	K00368	*nirK*	549.23	4210.61	3317.02	4355.83	890.57	3880.90	3580.00	6382.16
	1.7.2.7	K20932	*K20932*	-	-	-	-	-	-	-	-
	1.7.2.8	K20935	*hdh*	-	-	-	-	-	-	-	-

Table S5 The network analysis topology features between different groups.

Topological indices	F-A-P1	S-A-P1	F-O-P1	S-O-P1	F-A-P2	S-A-P2	F-O-P2	S-O-P2
Nodes	69	61	69	65	63	68	66	59
Links	58	54	51	50	63	45	60	59
Positive	36 (62%)	24 (44%)	28 (55%)	22 (44%)	28 (44%)	22 (49%)	29 (48%)	35 (59%)
Negative	22 (38%)	30 (56%)	23 (45%)	28 (56%)	35 (56%)	23 (51%)	31 (52%)	24 (41%)
Degree	1.681	1.770	1.478	1.538	2.000	1.324	1.818	2.000
Diameter	3	3	2	3	3	3	3	3
Density	0.025	0.030	0.022	0.024	0.032	0.020	0.028	0.034
Clustering coefficient	0.787	0.865	0.806	0.806	0.857	0.682	0.863	0.952
Modularity	0.935	0.927	1.105	0.924	0.856	0.955	0.936	0.874

Table S6. Comparison of COD removal in FWW between HBR and other biological treatment processes.

Treatment process	Inlet COD concentration (mg/L)	COD load (kg/m³·d)	HRT (h)	Temperature (°C)	Effluent COD concentration (mg/L)	COD Removal rate (%)	Reference
MBR	1500 ~ 2000	3.4	15	22 ~ 23	< 70	96%	(Wang et al., 2005)
AnMBR	12770	4.52	60	37.0 ± 0.5	2388	80 ~ 85%	(He et al., 2005)
AnMBR	90200	9.3	/	39 ± 1	< 1600	> 98%	(Xiao et al., 2019)
AnDMBR	2050 ~ 6880	0.44	120	24 ± 2	100 ~ 200	94.5	(Galib et al., 2016)
HBR	29860 ± 276	2.67	268.84	22 ~ 23	900 ~ 1400	96.7 ~ 97.3%	This study
HBR	29860 ± 276	1.68	426.63	22 ~ 23	194 ~ 458	96.6 ~ 98.2%	This study

MBR: Membrane bioreactor; AMBR: Anaerobic membrane bioreactor; AnDMBR: Anaerobic dynamic membrane bioreactor; HBR: hybrid biofilm reactor.

Table S7 Contribution of column A and column O to COD removal in stage I.

Time (d)	COD volumetric load of O column	Contribution of column A (%)	Contribution of column O (%)	Total COD removal rate (%)
D_{51}	1.82	29.38	68.19	97.57
D_{52}	2.06	20.27	77.31	97.59
D_{53}	2.07	19.19	77.82	97.02
D_{54}	2.49	3.83	93.30	97.13
D_{55}	2.32	9.13	86.98	96.10
D_{56}	2.35	7.61	88.05	95.66
D_{57}	2.31	7.88	86.72	94.60
D_{58}	2.57	-2.01	96.59	94.58
D_{59}	2.25	7.21	84.39	91.60
D_{60}	2.53	1.62	94.78	96.40
D_{61}	2.10	15.99	78.77	94.76
D_{62}	2.45	4.91	92.00	96.91
D_{63}	2.17	14.02	81.30	95.32
D_{64}	2.21	12.74	82.93	95.66
D_{65}	2.08	18.83	77.90	96.73
D_{66}	2.17	14.39	81.48	95.87
D_{67}	2.18	13.80	81.84	95.64
D_{68}	2.18	13.67	81.66	95.33
D_{69}	2.10	17.18	78.93	96.11
D_{70}	1.96	22.29	73.61	95.89
D_{71}	1.85	24.87	69.40	94.27
D_{72}	1.71	30.52	63.98	94.49
D_{73}	1.97	20.79	73.97	94.76
D_{74}	1.88	24.54	70.68	95.23
D_{75}	1.93	25.58	72.33	97.91
Mean	**2.15**	**15.13**	**80.60**	**95.73**

Table S8 Performance of UASB process in degrading organic wastewater.

Wastewater types	Inlet COD concentration (mg/L)	Inlet COD load (kg/m^3·d)	Temperature (°C)	Reactor volume (m^3)	COD Removal rate (%)
Alcohol wastewater	34060	13.6	32	130	81
Brewing wastewater	2000 ~ 6000	4.2	Ambient	64.8	82.4
Slaughterhouse wastewater	1000	2.5	Ambient	250	77
Acetone butanol wastewater	15000	2.5	35	3000*2	80
Fermented drug mixture	23450	11.8	35	200*4	91
Sugar fermentation wastewater	20000	4	35	-	85
Citric acid wastewater	10000	10	35	380	80

Table S9 Permissible organic volume loading rate of different wastewater at different temperatures.

Temperature (℃)	Organic volume loading rate (kg COD /m³·d)		
	VFA containing wastewater	VFA free wastewater	Wastewater with SS accounting for 30% of COD
15	2 ~ 4	1.5 ~ 3	1.5 ~ 2
20	4 ~ 6	2 ~ 4	2 ~ 3
25	6 ~ 12	4 ~ 8	3 ~ 6
30	10 ~ 18	8 ~ 12	6 ~ 9
35	15 ~ 24	12 ~ 18	9 ~ 14
40	20 ~ 32	15 ~ 24	14 ~ 18

Data sourced from: Hu JC, Zhou MJ, Zuo JE, Zhou Qi, He M. Theory and technology of anaerobic biological treatment of wastewater[M], China Architecture Publishing & Media Co., Ltd. 2003: 1-303. (In Chinese) (VFA: volatile fatty acids, SS: suspended solid)

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