1 **Supporting Information**

2 **Bacterial and microfauna mechanisms for sludge reduction in carrier-enhanced**

3 **anaerobic side-stream reactor revealed by metagenomic sequencing analysis**

4 Jie Jiang[a], Zhen Zhou[a,c,*], Lingyan Jiang[b,c], Yue Zheng[a], Xiaodan Zhao[a], Guang Chen[b],

5 Mengyu Wang[a], Jing Huang[a], Ying An[a], Zhichao Wu[c,d]

6 [a] Shanghai Engineering Research Center of Energy - Saving in Heat Exchange Systems,

7 Shanghai University of Electric Power, Shanghai 200090, China

8 [b] Shanghai Chengtou Wastewater Treatment Co., Ltd, Shanghai 201203, China

9 [c] Shanghai Institute of Pollution Control and Ecological Security, Shanghai, 200092,

10 China

11 [d] State Key Laboratory of Pollution Control and Resource Reuse, College of

12 Environmental Science and Engineering, Tongji University, Shanghai, 200092, China

13 * Corresponding author. E-mail: zhouzhen@shiep.edu.cn.

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1. Supplementary methods

Text S1

1.1. Calculation methods for Y_{obs} and K_d

The observed sludge yield (Y_{obs}) was calculated according to Eq. (1) [1].

$$Y_{obs} = \frac{\sum \Delta X_i V_i + \sum Q_w X_w \Delta t}{\sum Q_i (S_0 - S_e) \Delta t} \tag{1}$$

where ΔX_i is the varied sludge concentration in reactor i, mg/L; Δt is duration of the operation period, d; V_i is the volume of reactor i, L; Q_w is flow rate of WAS, L/d; X_w is sludge concentration in WAS, mg/L; S_0 and S_e is substrate concentration of influent and effluent, respectively, mg COD/L.

At a steady state, sludge decay in an ASSR-MBR or its deviation can be expressed as Eq. (2) according to mass balance model [2].

$$\Delta X_{d,m} + \Delta X_{d,a} = K_{d,m} V_m X_{s,m} + K_{d,a} V_a X_{s,a} = \Delta X_{g,m} + \Delta X_{g,a} - \Delta X_w \tag{2}$$

where $\Delta X_{d,m}$ and $\Delta X_{d,a}$ are sludge decay in the main stream (using subscript 'm') and ASSR (using subscript 'a'), g/d; ΔX_w is sludge variation item of the system, including WAS discharge, effluent loss and sludge accumulation in reactors, g/d; $\Delta X_{g,m}$ and $\Delta X_{g,a}$ are sludge generated in the main stream and ASSR, g/d; $K_{d,a}$ and $K_{d,m}$ are decay coefficients of ASSR and the main stream reactor, d^{-1}; V_a and V_m are volumes of ASSR and the main stream reactor, L; $X_{s,a}$ and $X_{s,m}$ are sludge concentrations in ASSR and the main stream reactor, g/L. Items $\Delta X_{g,m}$ and $\Delta X_{g,a}$ can be calculated based on biomass growth in the main stream and ASSR according to methods and parameters recommended in literatures [2, 3]. Then $K_{d,m}$ and $K_{d,a}$ can be estimated by substituting

41      $\Delta X_{g,m}$ and $\Delta X_{g,a}$ in Eq. (2).

42   Text S2:

43   *1.2. Microbial community analysis*

44   The samples were first processed for DNA extraction using E.Z.N.A. ®. Soil DNA

45   Kit for soil (OMEGA, USA) according to manufacturer's protocols. The quantity and

46   quality of the extracted DNA were assessed using a Nanodrop ND-1000

47   spectrophotometer (Labtech International, UK). Low absorption ratios at 260/230 and

48   260/280 nm were used as an indicator of humic acids, polysaccharides and protein

49   impurities. The v3-v4 region of the 16S rRNA gene was amplified by PCR using 338F

50   (5'-ACTCCTACGGGAGGCAGCAG-3')   and   806R   (5'-

51   GGACTACHVGGGTWTCTA AT-3'). PCR products of the same sample were mixed

52   and recovered using 2% agarose Gel. The products were purified using the AxyPrep

53   DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). The products

54   were tested by 2% agarose Gel electroencephalography and quantified using Quantus™

55   Fluorometer (Promega, USA). NEXTFLEX Rapid dna-seq Kit was used to build the

56   library. Sequencing was performed using the *Illumina-Miseq* PE300 platform. Then

57   16S rRNA sequences were clustered into operational taxonomic units with an average

58   length of 440 bp by setting a 3% distance limit[4].

59   Text S3:

60   *1.3. Mass of the biofilms on the carriers and the surface of membrane*

61   The biomass of the biofilms was determined on carriers from the stable operating

62   reactor. For each measurement, ten carriers covered by biofilm were taken out of the

63   reactor by five-point sampling. Then the carriers were dried over night at 105 ℃ and

64   weighed, after which the biofilm was removed by washing of wire cleaning brush in 1

65   M NaOH and deionized water. The clean carriers were again dried and weighed.

66   Biofilm biomass was calculated as the difference in dry weight before and after cleaning.

67   The tests of biofilm biomass were performed in duplicate.

68   The petri dishes were dried at 105 ℃ in advance. The sludge on the surface of

69   membrane was scraped off and put into the petri dishes, and dried at 105℃ for 12 h.

70   SS on the surface of membrane was calculated as the difference in petri dishes before

71   and after putting into the sludge.

72   Text S4:

73   1.4 Mass balance model for intrasystem flow of nitrogen

74   Mass balance was constructed to describe mitigation and transformation of nitrogen

75   in water, sludge and air pathways based on experimental data obtained from the four

76   pilot-scale systems. Mass balance model of nitrogen was established according to

77   Huang, et al. [5]

78 **2. Supplementary Results**
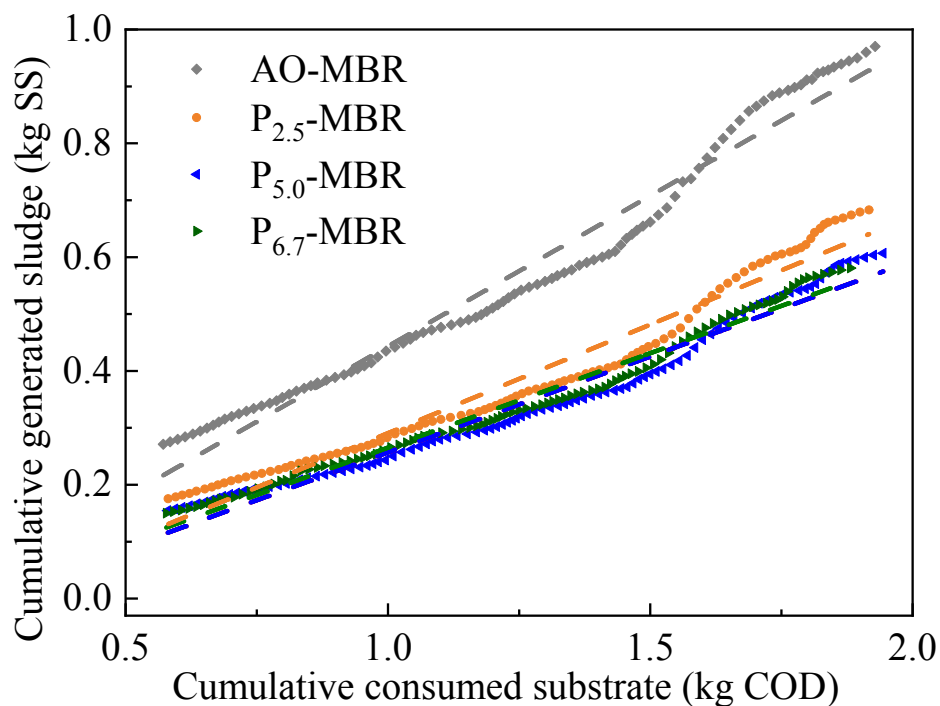
80   **Figure S1** Observed sludge yield in ASSRsAO-MBR and AP-MBRs.
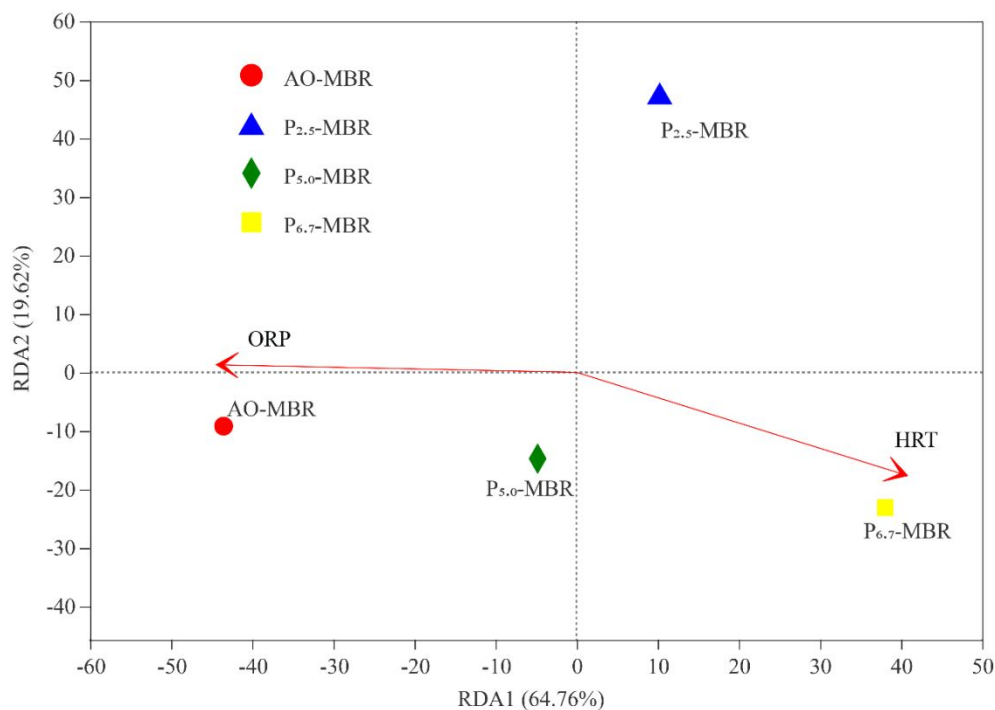
82   **Figure S2** Redundancy analysis (RDA) analysis of *MiSeq* data (symbols) and

83   environmental characteristics (arrows).
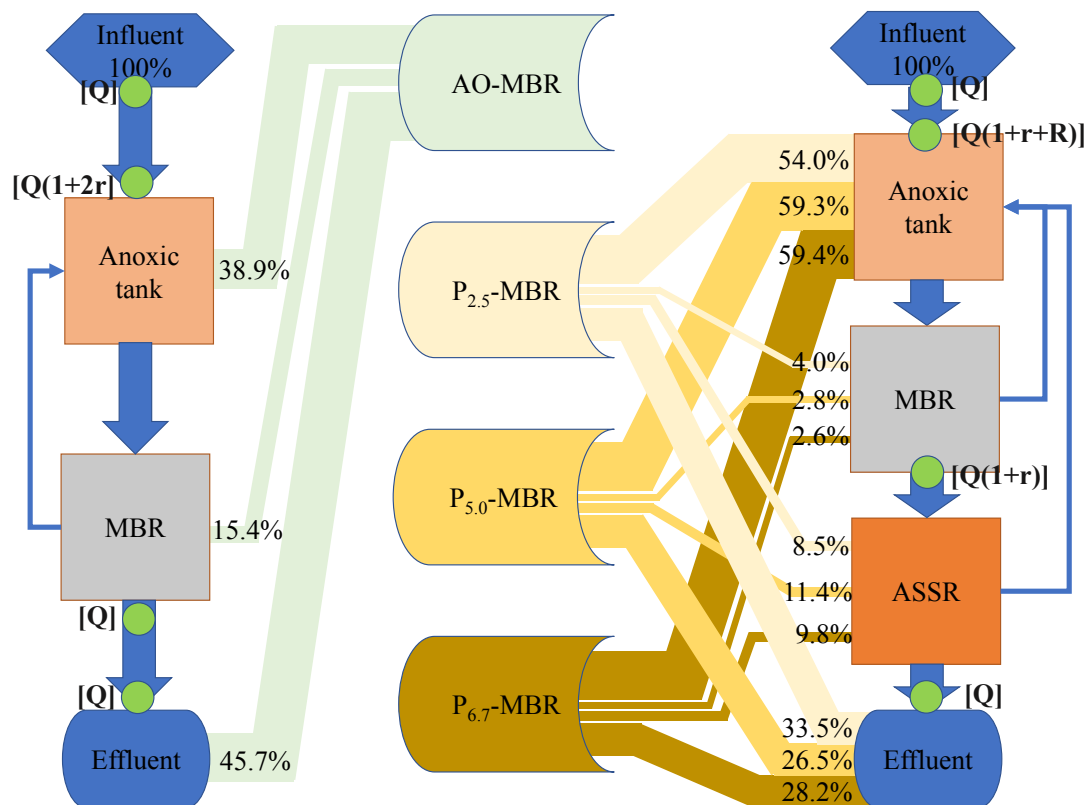
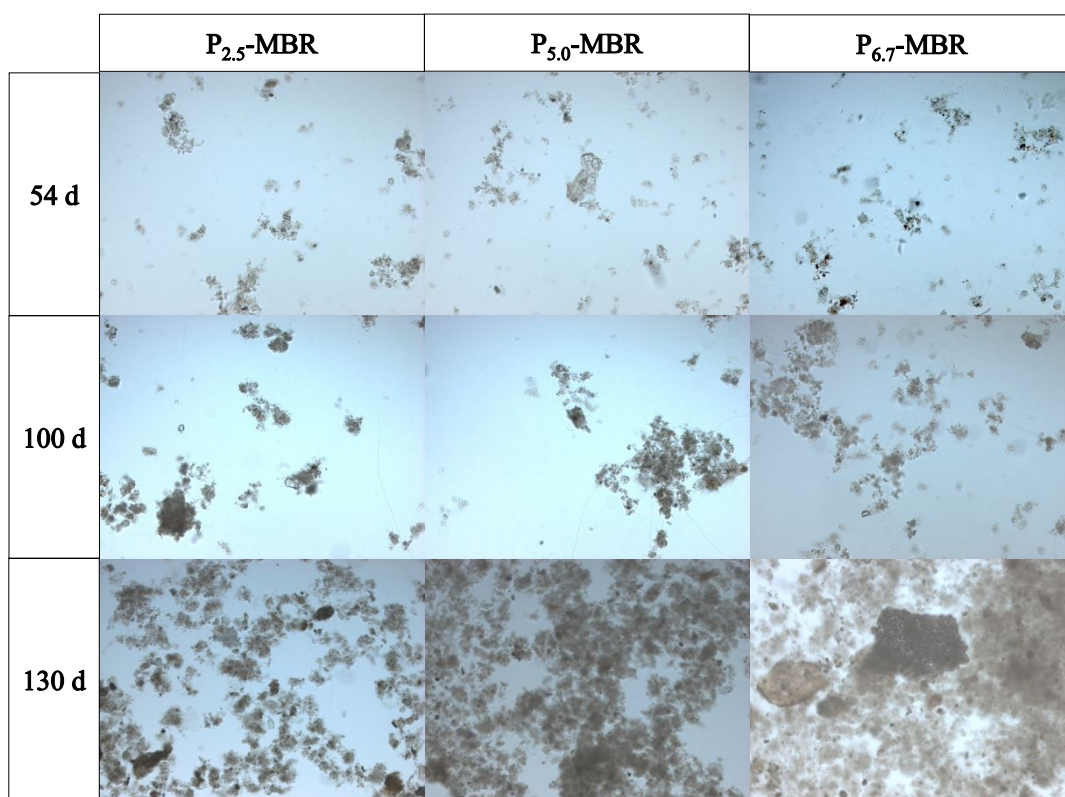**Figure S3** Mass balance of nitrogen in AO-MBR and AP-MBRs.

Figure S4 Microscope images of the biofilm on the carriers.

88 **Table S1** COD consumption in ASSR by ΔCOD_o, ΔCOD_d and ΔCOD_c with different

89 HRT_{SR}

HRT_{SR}	ΔCOD_o (mg/L)	ΔCOD_d (mg/L)	ΔCOD_c (mg/L)	Total (mg/L)
2.5	12.36	19.38	7.00	38.74
5	12.73	21.98	9.60	44.31
6.7	12.58	22.09	10.52	45.19

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91 **Table S2** Statistics and assembly results of the metagenomic sequencing data

Samples	Raw sequence			Sequence after quality-filtering				Assembly		
	Raw reads $(\times 10^7)$	Raw base $(\times 10^9 \text{ bp})$	Clean reads $(\times 10^7)$	Clean base $(\times 10^9 \text{ bp})$	Percent of raw reads (%)	Percent of raw bases (%)		Contigs $(\times 10^5)$	N50 (bp)	N90 (bp)
MBR_{AO}	5.48	8.27	5.41	8.16	98.8	98.6		8.60	640	345
$ASSR_L$	4.23	6.39	4.20	6.33	99.2	99.0		7.09	716	351
$ASSR_M$	5.10	7.70	5.07	7.64	99.4	99.2		8.61	703	351
$ASSR_H$	5.29	7.99	5.26	7.93	99.4	99.2		7.96	702	351
P_L	5.79	8.74	5.75	8.66	99.4	99.1		6.09	703	350
P_M	6.20	9.36	6.16	9.29	99.4	99.2		7.28	689	347
P_H	5.81	8.78	5.78	8.7	99.3	99.1		7.47	730	353

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93   **Table S3** Population distributions of protozoa and metazoa expressed in abundance categories

Kingdom	Class	Species	Operation Time (d)	MBRs				ASSRs			Packing Carriers		
				MBR$_{AO}$	MBR$_L$	MBR$_M$	MBR$_H$	ASSR$_L$	ASSR$_M$	ASSR$_H$	P$_L$	P$_M$	P$_H$
Protozoa	Sarcodine	*Arcella*	54				++			+++			+++
			100	+	+	+++	++	+	+++	++			+++
			130	+	+	+++	+++	+	+++	+++		+++	+++
		Clathrulina	54										
			100		++	+++	+++	+	+++	+++			
			130		++	++	+	+	++	+++			++
	Ciliate	*Aspidisca*	54	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
			100	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
			130	+++	+++	+++	+++	+++	+++	+++	++	+++	+++
		Litonotus	54			+++	+++	++	+++	+++			+++
			100			+++	+++	+	+++	+++	+++	+++	+++
			130			+++	+++	++	+++	+++	+++	+++	+++
		Chiloclonella	54		+++	+++	+++	++	+++	+++		+	

	100	+	+++	+++	+++	+++	+++	+++	+	++	+++
	130		+++	+++	+++	+++	+++	+++	++	+++	+++
	54			+	+						
Tokophrya	100			+	+						
	130			+	++						
	54										+
Heliophrya	100										+
	130									+	+
	54						+	+			
Cothumia	100	+++		+	+	+	+	+	+	+	+
	130										
	54	+				+					
Paramecium	100	+				++					
	130										
	54							+			+++
Scyphidia	100			+	+						
	130										

			54										++
		Stentor	100										
			130										
			54			+	+					+	+++
		Vorticella	100			+++	+++				+++	+++	+++
			130		++	+++	+++	++	++	++	+	+	++
			54										
		Epystilis	100										
			130	+	+	++	+++						
			54							+			+++
		Rotaria	100	++				+	++	+++			+++
	Rotifer		130	++++	++++	++++	++++	+	+++	+++	+++	+++	+++
Metazoa			54										
		Adineta	100	+			+++						
			130	+	+++	++++	++++						
	Oligotrichia	*Aelosoma*	54			+							
		Hemproehi	100							+			

		130								++	++		
		54										+	
Crustacean	*Cylops*	100	+									+	+++
		130										++	++++
		54											
Nematoda	*Nematoda*	100											
		130	+	+	+	+	+	+	+	+++	+++	+++

94   Note: 1. Absence: (blank); scarce: 1-50 ind./mL (+); moderate: 51–100 ind./mL (++); abundant: 101–500 ind./mL (+++); more abundant: >500 ind./mL (++++).

95   2. The species and abundance of microfauna in the inoculated sludge were sarcodine (+) and rotifers (+) with small size.

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