

Supplemental Materials

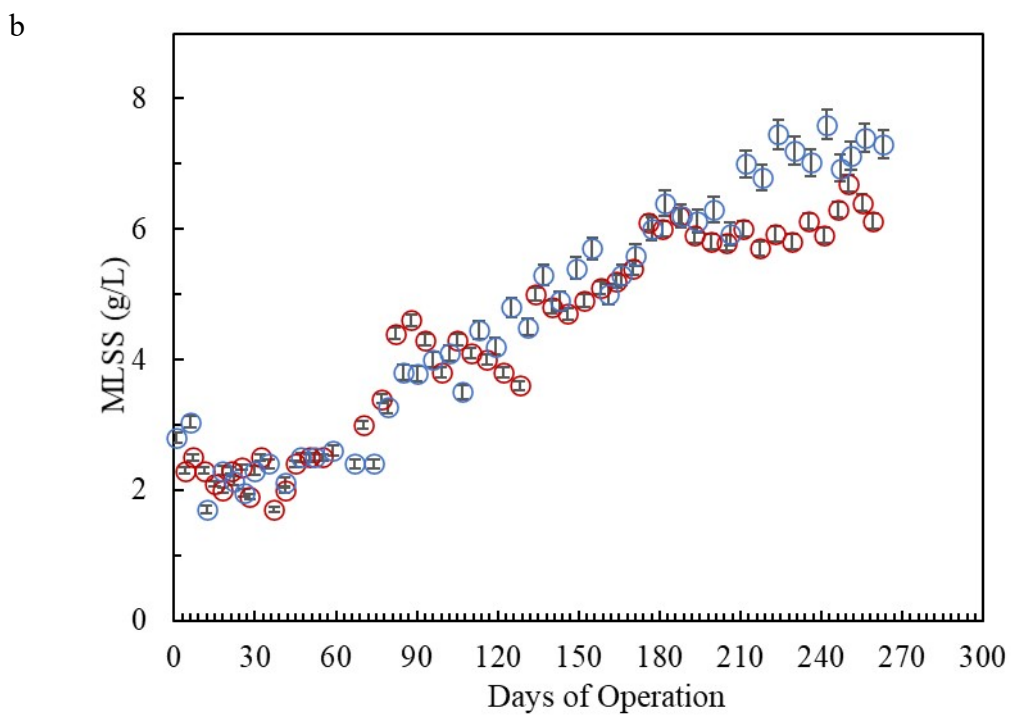
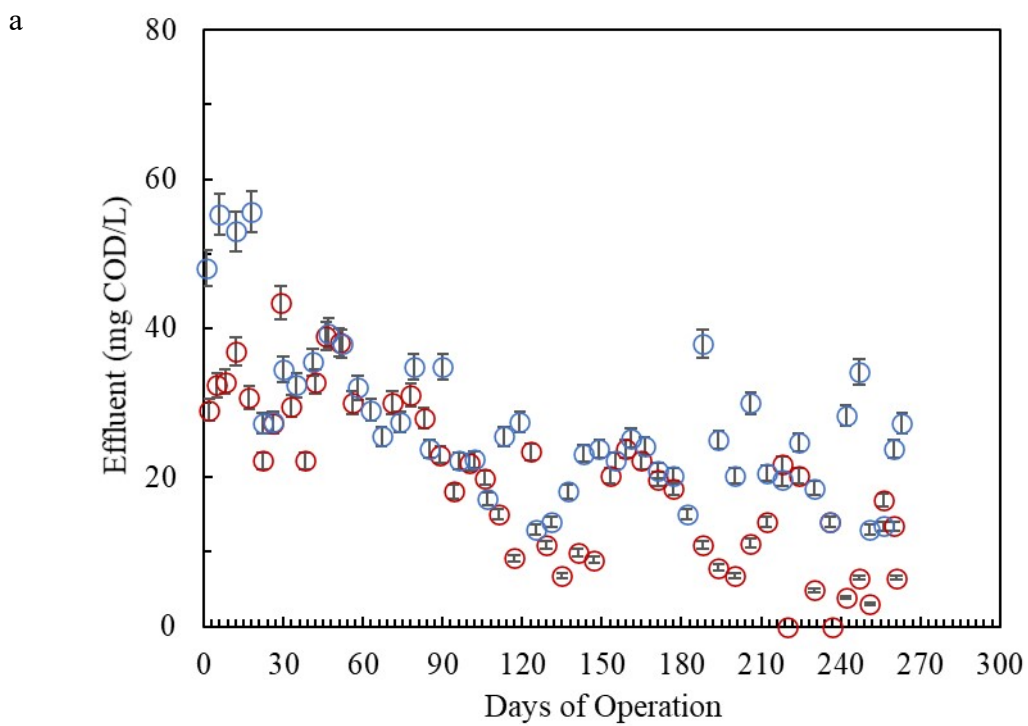


Figure S1 Effluent COD (a) and MLSS (b) concentrations from the two AnMBRs (red circles for AnMBR-A; blue circles for AnMBR-G) at decreasing HRTs (24 h from day 1 to day 60, 12 h from day 61 to day 170, and 6 h from day 170 to day 270).

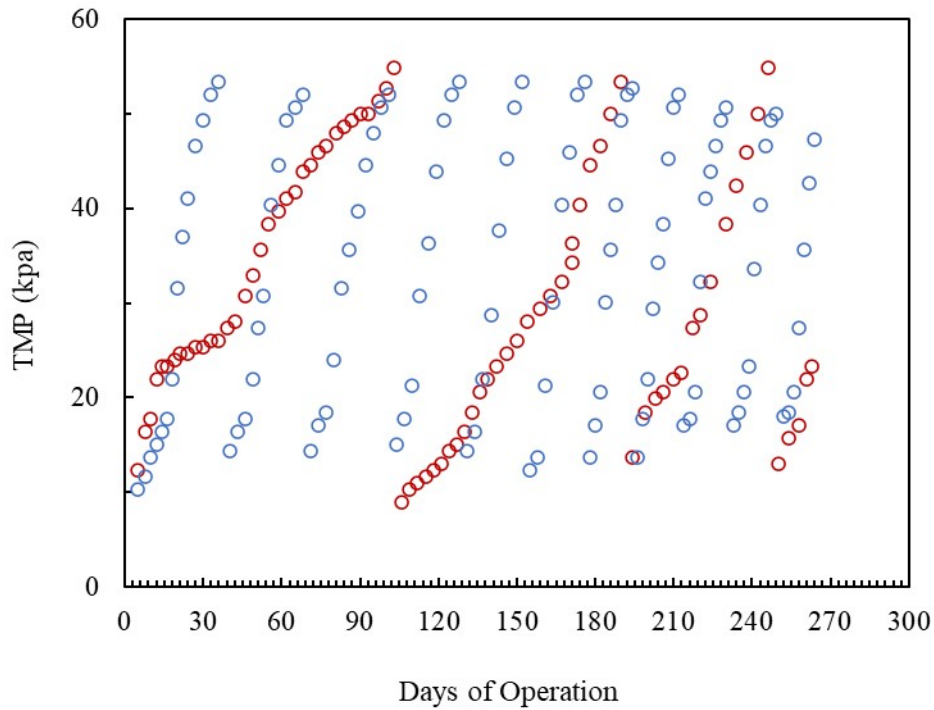


Figure S2 Changes in TMP with time from the two AnMBRs (red circles for AnMBR-A; blue circles for AnMBR-G) over a period of approximately 270 days.

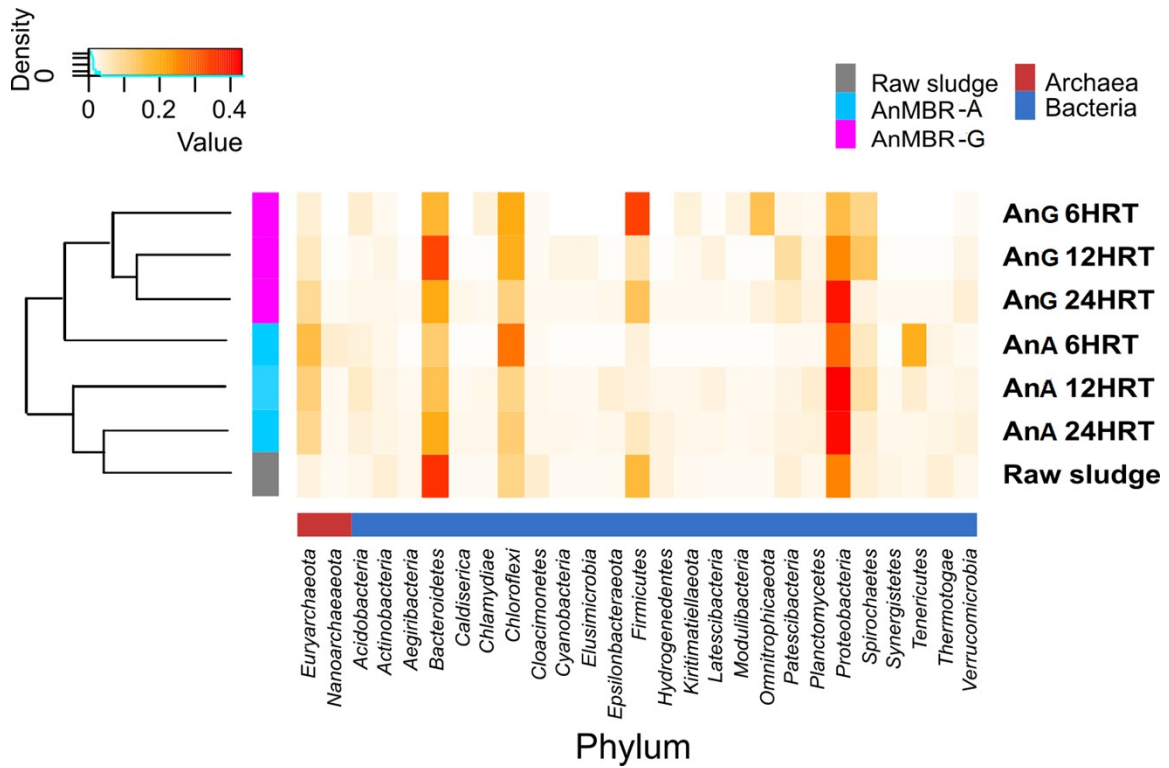


Figure S3 Heatmap of the distribution of dominant phyla and Joining clustering by using Bray-Curtis similarity index of anaerobic sludge obtaining from the AnMBRs (AnA stands for AnMBR-A and AnG stands for AnMBR-G) at decreasing HRTs.

Table S1 Operating conditions of AnMBR systems.

Treatment	AnMBR-A			AnMBR-G		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
Carbon Source	Sodium acetate			Glucose		
HRT (h)	24	12	6	24	12	6
SRT (d)	40					

Table S2 Significance tests of the structures of the whole communities between samples obtaining from the AnMBRs at decreasing HRTs (from 24, to 12, and to 6 h).

Sludge samples	Raw sludge	24 h	12 h	6 h
AnMBR-A	24 h	$p < 0.01$		$p < 0.01$
	12 h	$p < 0.01$	$p < 0.01$	$p < 0.01$
	6 h	$p < 0.01$	$p < 0.01$	$p < 0.01$
AnMBR-G	24 h	$p < 0.01$		$p < 0.01$
	12 h	$p < 0.01$	$p < 0.01$	$p < 0.05$
	6 h	$p < 0.01$	$p < 0.01$	$p < 0.05$

Table S3 Significance tests of the structures of the whole communities between samples obtaining from each reactor in the middle and end of each operational stage.

Treatment		AnMBR-A	
		Middle*	End*
24 h	Middle		$p > 0.05$
	End	$p > 0.05$	
12 h	Middle		$p > 0.05$
	End	$p > 0.05$	
6 h	Middle		$p > 0.05$
	End	$p > 0.05$	
		AnMBR-G	
24 h	Middle		$p > 0.05$
	End	$p > 0.05$	
12 h	Middle		$p > 0.05$
	End	$p > 0.05$	
6 h	Middle		$p > 0.05$
	End	$p > 0.05$	

Middle*: Sludge obtaining in the middle of the run

End*: Sludge obtaining at the end of the run

Table S4 Conversion of the average copies/L to cells/L, cells/g, and percent of dominant species in anaerobic sludge obtained from AnMBRs at decreasing HRTs.

		AnMBR-A				AnMBR-G			
		copies/L	cells/L _a	cells/g ^b	% Biomass ^c	copies/L	cells/L _a	cells/g ^b	% Biomass ^c
Raw sludge	Bacteria	2.07E+1 1	5.19E+ 10	3.99E+ 10	78.52	2.07E+1 1	5.19E+ 10	3.99E+ 10	78.52
	Archaea	2.33E+1 0	1.42E+ 10	1.09E+ 10	21.48	2.33E+1 0	1.42E+ 10	1.09E+ 10	21.48
	<i>Methanosaeta</i>	1.33E+1 0	8.85E+ 09	6.81E+ 09	13.39	1.33E+1 0	8.85E+ 09	6.81E+ 09	13.39
	<i>Methanosarcina</i>	6.78E+0 8	2.26E+ 08	1.74E+ 08	0.34	6.78E+0 8	2.26E+ 08	1.74E+ 08	0.34
	<i>Methanomicrobiales</i>	4.90E+0 9	2.72E+ 09	2.09E+ 09	4.12	4.90E+0 9	2.72E+ 09	2.09E+ 09	4.12
	<i>Methanobacteriales</i>	1.02E+0 8	4.61E+ 07	3.55E+ 07	0.07	1.02E+0 8	4.61E+ 07	3.55E+ 07	0.07
			copies/L	cells/L	cells/g	% Biomass	copies/L	cells/L	cells/g
Sludge at HRT of 24 h	Bacteria	2.46E+1 1	6.17E+ 10	2.80E+ 10	59.32	3.61E+1 1	9.08E+ 10	3.95E+ 10	51.18
	Archaea	6.94E+1 0	4.23E+ 10	1.92E+ 10	40.68	1.42E+1 1	8.66E+ 10	3.76E+ 10	48.82
	<i>Methanosaeta</i>	5.78E+1 0	3.85E+ 10	1.59E+ 10	33.67	4.40E+1 0	2.94E+ 10	1.28E+ 10	16.55
	<i>Methanosarcina</i>	1.47E+0 9	4.88E+ 08	2.22E+ 08	0.47	1.88E+0 9	6.27E+ 08	2.72E+ 08	0.35
	<i>Methanomicrobiales</i>	1.96E+1 0	1.09E+ 10	4.51E+ 09	9.54	3.28E+1 0	1.82E+ 10	7.92E+ 09	10.28
	<i>Methanobacteriales</i>	6.83E+0 7	3.10E+ 07	1.41E+ 07	0.03	2.49E+1 0	1.13E+ 10	4.92E+ 09	6.38
			copies/L	cells/L	cells/g	% Biomass	copies/L	cells/L	cells/g
Sludge at HRT of 12 h	Bacteria	2.54E+1 1	6.39E+ 10	1.49E+ 10	40.51	5.90E+1 1	1.48E+ 11	3.37E+ 10	78.67
	Archaea	1.54E+1 1	9.38E+ 10	2.18E+ 10	59.49	6.60E+1 0	4.02E+ 10	9.14E+ 09	21.33
	<i>Methanosaeta</i>	1.32E+1 1	8.82E+ 10	2.05E+ 10	55.59	4.14E+1 0	2.76E+ 10	6.27E+ 09	14.63
	<i>Methanosarcina</i>	6.74E+0 9	2.25E+ 09	5.22E+ 08	1.43	3.17E+0 9	1.06E+ 09	2.40E+ 08	0.56
	<i>Methanomicrobiales</i>	5.65E+0 9	3.14E+ 09	7.30E+ 09	1.99	1.05E+1 0	5.84E+ 09	1.33E+ 09	3.10
	<i>Methanobacteriales</i>	1.38E+0 8	6.27E+ 07	1.46E+ 07	0.04	2.01E+1 0	9.15E+ 09	2.08E+ 09	4.85
			copies/L	cells/L	cells/g	% Biomass	copies/L	cells/L	cells/g
Sludge at HRT of 6 h	Bacteria	2.58E+1 1	6.48E+ 10	1.62E+ 10	37.57	5.20E+1 0	1.31E+ 10	1.00E+ 10	82.81
	Archaea	1.77E+1	1.08E+	2.69E+	62.43	4.45E+0	2.71E+	2.08E+	17.19

	1	11	10		9	09	09	
<i>Methanosaeta</i>	1.58E+1	1.05E+	2.63E+	61.08	2.98E+0	1.99E+	1.53E+	
	1	11	10		9	09	09	12.60
<i>Methanosarcin</i>	9.14E+0	3.05E+	7.62E+	1.77	1.17E+0	3.90E+	3.00E+	
<i>a</i>	9	09	08		8	07	07	0.25
<i>Methanomicro</i>	2.55E+0	1.42E+	3.54E+	0.82	1.08E+0	6.00E+	4.62E+	
<i>biales</i>	9	09	08		9	08	08	3.81
<i>Methanobacter</i>	2.19E+0	9.95E+	2.49E+	0.06	1.75E+0	7.95E+	6.12E+	
<i>iales</i>	8	07	07		9	08	08	5.05

Values are averaged for samples.

^a Cells/L = copies/L ÷ gene copy number/cell. (The average 16S rDNA copies per genome in bacteria, archaea, *Methanosaeta*, *Methanosarcina*, *Methanomicrobiales*, and *Methanobacteriales* was 3.98, 1.64, 1.5, 3, 1.8, and 2.2, repetitively, according to previous studies (Hou et al., 2020; Klappenbach et al., 2001))

^b Cells/g = cells/L ÷ g/L (mean mixed liquor suspended solids (MLSS)).

^c The total of Bacterial and Archaeal 16S rRNA gene provides normalization for comparison to the subpopulations and is set at 100%.

References

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<http://doi.org/10.1093/nar/29.1.181>