

Electronic Supplementary Information

Microbial community and antibiotic resistance profiles of biomass and effluent are distinctly affected by antibiotic addition to an anaerobic membrane bioreactor

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Quantification of antibiotics by LC-MS

For antibiotics quantification, 10 mL samples were collected for each sampling time point from the influent and effluent of the AnMBR. Both collected samples and standard solutions were filtered through 0.2 µm PTFE syringe filters (Whatman) using 10 mL syringes with Luer lock tips and stored in certified 2 mL amber LC vials (Agilent) at 4 °C refrigerator for no more than 3 days prior to analysis. Stock solutions of sulfamethoxazole and erythromycin were prepared in HPLC-grade methanol at concentrations of 20 mg/L and stored at -20 °C. Ampicillin stock solution was prepared in HPLC-grade water at 4 mg/L due to its lack of solubility in methanol and stored at 4 °C. For each antibiotic, a six-point standard calibration curve was constructed within the appropriate range (i.e., 0.1-30 µg/L to target effluent antibiotics and 30-400 µg/L to target influent antibiotics). All calibration curve R^2 values were above 0.99. Both solvent-based and matrix-matched calibration curves were generated for all three compounds to ensure that the solvent based standards were representative of influent and effluent concentrations.

Positive ESI MS-Q-TOF mode was employed to target sulfamethoxazole, erythromycin, and ampicillin. The LC gradient program for detection of all three compounds utilized 0.1% formic acid in water as mobile phase A and acetonitrile as mobile phase B as follows: t=0.0 min A=90% B=10%, t=3.0 min A=0% B=100%, t=5.0 min A=0% B=100%, t=5.10 min A=90% B=10%. LC conditions used included a flow rate of 0.4 mL/min, maximum pressure of 600 bar, column temperature of 40 °C, and autosampler tray temperature of 8 °C. A post-column switch was used to divert the first 0.5 min of column elution to waste to avoid sending hydrophilic compounds from the effluent matrix through the MS. Injection volumes ranged from 0.5-10 µL, depending on the target sample range (influent and effluent) for each operational phase, to ensure that no compound extracted ion chromatogram peaks exceeded saturation detection values. MS conditions used were as follows: sheath gas temp. of 400 °C, sheath gas flow rate of 12 L/min, gas temperature of 225 °C, drying gas flow rate of 5 L/min, nebulizer pressure of 20 psi, capillary voltage of 3500V, nozzle voltage of 500V, acquisition rate of 1.5 spectra/s, and acquisition time of 667 ms/spectrum. Targeted compound acquisition parameters are provided in Table S1. All compound detection and quantification analyses were performed using the Agilent MassHunter Qualitative Analysis Navigator program.

Table S1. Targeted antibiotic properties and MS data acquisition parameters.

Compound	Molecular Weight (MW)	Retention Time (min)	MS Spectrum (m/z)	Fragmentor Voltage (V)
Sulfamethoxazole	253.052	2.17	254.059	400
Erythromycin	733.461	2.35	734.469	100
Ampicillin	349.110	1.44	350.117	400

Table S2. Synthetic wastewater composition.

Concentrate solution		Dilution water	
Reagent	Concentration (mg/L)	Reagent	Concentration (mg/L)
Ammonium Chloride	11.5	Sodium Bicarbonate	369
Calcium Chloride	11.5	Magnesium Phosphate	30.8
Iron Sulfate	7.7	Potassium Phosphate	13.8
Sodium Sulfate	11.5	Sodium Hydroxide	18.5
Sodium Acetate	27		
Urea	87		
Peptone	11.5		
Yeast	46		
Milk Powder	115.4		
Soy Oil	13.5		
Hydrochloric Acid	0.2		
Starch	115.4		
Chromium Nitrate	3.7		
Copper Chloride	2.5		
Manganese Sulfate	4.9		
Nickel Sulfate	1.2		
Lead Chloride	0.5		
Zinc Chloride	1.2		

Table S3. Forward and reverse primers and qPCR thermocycling conditions of all ARGs, *intl1*, and *rpoB* gene.

Gene	Primers	Preincubation	Amplification	Melting	Amplification size (bp)	Detection limit (gene copy/mL)	Ref.
ermF	F- CGACACAGCTTTGGTTGAAC R- GGACCTACCTCATAGACAAG	95° for 900 s	40 cycles, 95° for 30 s, 56° for 60 s, 70° for 60	95° for 10 s, 65° for 60 s, 97° for 1 s	309	350	1
ermB	F- GATACCGTTTACGAAATTGG R-GAATCGAGACTTGAGTGTGC	95° for 300 s	45 cycles, 95° for 30 s, 58° for 30 s, 72° for 30 s	95° for 10 s, 65° for 60 s, 97° for 1 s	364	1701	1
sul1	F- CGCACCGGAAACATCGTGCAC R- TGAAGTTCCGCCCAAGGCTCG	95° for 300 s	40 cycles, 95° for 15 s, 58° for 30 s, 72° for 30 s	95° for 10 s, 65° for 60 s, 97° for 1 s	163	155	2
sul2	F- TCCGGTGGAGGCCGGTATCTGG R- CGGGAATGCCATCTGCCTTGAG	95° for 900 s	50 cycles, 95° for 15 s, 58° for 30 s, 72° for 30 s	95° for 10 s, 65° for 60 s, 97° for 1 s	191	821	2
intl1	F- CTGGATTTGATCACGGCACG R- ACATGCGTGTAATCATCGTCCG	95° for 900 s	45 cycles, 95° for 30 s, 60° for 60 s	95° for 10 s, 65° for 60 s, 97° for 1 s	196	159	3
oxa-1	F- TATCTACAGCAGCGCCAGTG R- CGCATCAAATGCCATAAGTG	94° for 180 s	40 cycles, 94° for 30 s, 60° for 30 s, 72° for 60 s		199	12100	4
ampC	F- CCTCTTGCTCCACATTTGCT R- ACAACGTTTGCTGTGTGACG	95° for 300 s	45 cycles, 95° for 45 s, 58° for 45 s, 72° for 60 s	95° for 10 s, 65° for 60 s, 97° for 1 s	189	670	5
tetO	F- ACGGARAGTTTATTGTATACC R- TGGCGTATCTATAATGTTGAC	95° for 600 s	40 cycles, 95° for 15 s, 50° for 30 s, 72° for 30 s	95° for 10 s, 65° for 60 s, 97° for 1 s	171	2010	6
tetW	F- GAGAGCCTGCTATATGCCAGC R- GGGCGTATCCACAATGTTAAC	94° for 300 s	40 cycles, 94° for 30 s, 64° for 30 s, 72° for 30 s	95° for 10 s, 65° for 60 s, 97° for 1 s	168	1030	6
rpoB	F- AACATCGGTTTGTATCAAC R- CGTTGCATGTTGGTACCCAT	95° for 300 s	40 cycles, 95° for 30 s, 55° for 30 s, 72° for 30 s	95° for 10 s, 65° for 60 s, 97° for 1 s	381	5430	7

Table S4. Biomass ARG abundances (Copy/*rpoB*) in the pre-antibiotics, antibiotics loading, and post-antibiotics periods. Abundance and errors respectively represent the mean values and standard deviations calculated from triplicate qPCR results.

Day	<i>ermF</i>	<i>ermB</i>	<i>sul1</i>	<i>sul2</i>	<i>int11</i>	<i>oxa-1</i>	<i>ampC</i>	<i>tetO</i>	<i>tetW</i>
1	2.74 ±0.66 6 9	0.45 ±0.01 3 8	5.369 ±0.130	0.22 ±0.01 1 9	0.35 ±0.03 4 6	0.22 ±0.03 1 9	0.00 ±0.00 6 1	3.647 ±0.13 6	0.05 ±0.00 6 7
6	2.65 ±0.50 1 8	0.52 ±0.01 4 0	5.298 ±0.284	0.18 ±0.00 3 6	0.84 ±0.01 0 0	0.27 ±0.01 6 0	0.01 ±0.00 0 1	3.292 ±0.16 1	0.28 ±0.11 0 5
14	3.98 ±0.73 7 4	0.79 ±0.01 0 7	10.63 ±0.187 3	0.30 ±0.00 1 9	1.60 ±0.01 5 1	3.16 ±0.20 2 2	0.01 ±0.00 8 3	6.623 ±0.04 4	0.22 ±0.02 7 1
20	4.90 ±0.74 2 5	1.01 ±0.04 7 7	10.42 ±0.233 8	0.36 ±0.00 6 9	1.52 ±0.00 3 6	2.49 ±0.56 2 2	0.02 ±0.00 2 1	6.961 ±0.07 1	0.33 ±0.11 4 4
27	4.45 ±0.36 1 9	0.74 ±0.03 6 3	11.22 ±0.300 1	0.35 ±0.01 0 4	1.51 ±0.21 7 1	1.24 ±0.06 0 0	0.01 ±0.00 7 2	6.959 ±0.33 1	0.40 ±0.04 3 1
35	5.17 ±0.54 3 4	1.18 ±0.05 5 1	13.50 ±0.302 6	0.42 ±0.02 3 6	1.45 ±0.03 4 5	1.42 ±0.04 5 0	0.02 ±0.00 3 0	9.317 ±0.32 5	0.19 ±0.07 2 3
46	5.71 ±0.10 3 5	1.26 ±0.13 9 9	23.90 ±0.489 1	0.45 ±0.02 6 3	0.88 ±0.00 2 0	1.03 ±0.17 1 4	0.02 ±0.00 6 4	14.75 ±0.33 9 8	0.26 ±0.07 7 8

Table S5. Effluent ARG abundances (Copy/mL) in the pre-antibiotics, antibiotics loading, and post-antibiotics periods. Abundance and errors respectively represent the mean values and standard deviations calculated from triplicate qPCR results.

Day	<i>ermF</i>	<i>ermB</i>	<i>sul1</i>	<i>sul2</i>	<i>int11</i>	<i>oxa-1</i>	<i>ampC</i>	<i>tetO</i>	<i>tetW</i>	<i>rpoB</i>
1	1.9E+01 ±3.1E+00	6.1E+00 ±1.7E+00	3.8E+03 ±8.9E+01	1.9E+02 ±6.9E-01	4.9E+03 ±5.9E+01	4.7E+02 ±1.8E+01	4.4E+00 ±1.3E+00	1.4E+02 ±1.1E+01	3.6E+02 ±3.3E+01	5.1E+02 ±4.9E+00
6	1.6E+00 ±2.8E-01	5.8E+00 ±1.1E+00	2.6E+05 ±8.8E+02	1.9E+02 ±1.8E+00	7.9E+04 ±9.4E+02	5.3E+02 ±2.8E+01	1.4E+01 ±7.8E+00	3.2E+01 ±3.5E+00	4.7E+01 ±8.0E+00	5.0E+03 ±1.8E+02
14	3.0E+00 ±5.2E-01	6.8E+00 ±1.2E+00	3.5E+04 ±1.2E+03	6.1E+03 ±3.1E+02	2.1E+04 ±3.1E+03	6.7E+03 ±3.1E+02	2.0E+00 ±4.5E-02	7.0E+01 ±6.5E+00	1.7E+02 ±5.8E+01	2.5E+03 ±1.8E+02
20	9.8E-01 ±1.0E-01	9.1E+00 ±3.4E-01	9.8E+02 ±6.9E+01	7.7E+01 ±1.7E+01	5.2E+02 ±7.7E+01	3.2E+01 ±1.3E+01	2.1E+00 ±1.1E+00	2.2E+01 ±2.8E+00	9.8E-01 ±0.0E+00	8.9E+01 ±1.2E+01
27	6.6E-02 ±2.1E-02	7.2E+00 ±6.0E-01	1.2E+03 ±1.1E+02	1.4E+02 ±5.5E+00	7.9E+02 ±9.0E+01	3.2E+01 ±7.2E+00	2.6E+00 ±1.3E+00	1.9E+01 ±3.6E+00	1.1E+00 ±1.3E-01	7.3E+01 ±4.2E+00
35	1.0E+00 ±4.3E-02	1.2E+01 ±2.3E+00	2.8E+03 ±2.1E+02	9.6E+01 ±9.2E-01	2.3E+03 ±4.0E+02	8.7E+01 ±1.4E+01	1.8E+01 ±1.0E+00	2.6E+01 ±9.6E-01	2.1E+01 ±3.6E+00	1.2E+02 ±1.1E+01
46	8.6E+03 ±1.1E+03	1.0E+03 ±8.2E+01	1.5E+04 ±1.4E+02	6.6E+02 ±4.7E+00	1.5E+03 ±1.3E+02	5.8E+02 ±2.2E+02	0.0E+00 ±0.0E+00	8.6E+03 ±7.7E+02	2.6E+02 ±2.0E+02	2.3E+03 ±1.7E+02

Table S6. Correlation analysis results between the first 100 most abundant OTUs and ARGs in biomass and effluent of the AnMBR. Correlated ARGs had strong significant correlation ($p < 0.05$; and $p > 0.7$ or $p < -0.7$) with the assigned OTUs. Green rows show correlation in biomass samples and the yellow rows show correlation in effluent samples.

OUT	Kingdom	Phylum	Class	Order	Family	Genus	Correlated ARGs
Otu6	Bacteria	Bacteroidetes	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified	ermF, ermB, sul1, sul2, ampC, tetO
Otu8	Bacteria	Bacteroidetes	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified	int11, oxa-1
Otu9	Bacteria	Ignavibacteriae	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacteriaceae_unclassified	ermF, ermB, int11, ampC, oxa-1
Otu15	Bacteria	Chloroflexi	Chloroflexi_unclassified	Chloroflexi_unclassified	Chloroflexi_unclassified	Chloroflexi_unclassified	tetW
Otu16	Bacteria	Bacteroidetes	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified	ermB, sul1, sul2, tetO
Otu32	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_unclassified	Clostridiales_unclassified	ermF, ermB, sul1, sul2, ampC, tetO
Otu33	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Cloacibacterium	int11, oxa-1
Otu35	Bacteria	Verrucomicrobia	Subdivision3	Subdivision3_unclassified	Subdivision3_unclassified	Subdivision3_unclassified	ermF, ermB, sul1, sul2, ampC, tetO
Otu45	Bacteria	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	int11
Otu46	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Anaerolineaceae_unclassified	int11
Otu52	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Anaerolineaceae_unclassified	oxa-1, int11
Otu68	Bacteria	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	int11
Otu73	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Anaerolineaceae_unclassified	sul1, tetO
Otu74	Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae	Smithella	int11
Otu84	Bacteria	Firmicutes	Firmicutes_unclassified	Firmicutes_unclassified	Firmicutes_unclassified	Firmicutes_unclassified	ermF, ermB, sul1, sul2, ampC, tetO
Otu93	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Macellibacteroides	int11, oxa-1
Otu97	Bacteria	Verrucomicrobia	Subdivision3	Subdivision3_unclassified	Subdivision3_unclassified	Subdivision3_unclassified	ermB, sul1, sul2, tetO
Otu98	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Anaerolineaceae_unclassified	oxa-1, int11
Otu99	Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Aminivibrio	sul1, tetO
Otu105	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Anaerolineaceae_unclassified	ermF, ermB, sul1, sul2, ampC, tetO
Otu134	Bacteria	Bacteroidetes	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified	ermF, ermB, sul1, sul2, ampC, tetO
Otu135	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Anaerolineaceae_unclassified	int11

Otu168	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionales_unclassified	Desulfovibrionales_unclassified	sul1, tetO
Otu170	Bacteria	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	sul1, tetO
Otu171	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	sul1, tetO
Otu206	Bacteria	Ignavibacteriae	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacteriaceae_unclassified	sul2
Otu232	Bacteria	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	int11, oxa-1
Otu11	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerosinus	ermF, ermB, tetO
Otu20	Bacteria	Bacteroidetes	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified	sul1, int11
Otu21	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Propionispira	ermF, ermB, tetO
Otu25	Bacteria	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	sul1, int11
Otu27	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Arcobacter	sul1, int11
Otu48	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Dechloromonas	ermF, ermB, tetO
Otu60	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae_unclassified	sul2, oxa-1
Otu66	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Novispirillum	sul1, int11
Otu70	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	sul2, oxa-1
Otu78	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonas	sul1, int11
Otu89	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	sul1, int11
Otu92	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified	tetW
Otu111	Bacteria	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	sul2, oxa-1
Otu159	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Acetobacteroides	sul1, int11
Otu163	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	Methylophilus	sul2, oxa-1
Otu175	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Sulfurospirillum	tetW
Otu178	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Rhodocyclaceae_unclassified	ermF, ermB, tetO
Otu184	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonas	ermF, ermB, tetO
Otu209	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfobulbus	sul2, oxa-1
Otu247	Bacteria	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	sul1, int11
Otu264	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	sul1, int11
Otu287	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	sul2, oxa-1
Otu299	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Rhodopseudomonas	ermF, ermB, tetO
Otu352	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	sul1, int11
Otu360	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Cryomorphaceae_unclassified	sul2, oxa-1
Otu372	Bacteria	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	sul1, int11
Otu384	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Spirochaetaceae_unclassified	sul2, oxa-1
Otu387	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter	tetW

Otu389	Bacteria	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	ermF, ermB, tetO
Otu441	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	Sulfuricurvum	sul1, int1
Otu499	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bosea	sul2, oxa-1
Otu501	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacteraceae_unclassified	sul1, int1
Otu550	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Chryseobacterium	sul1, int1
Otu648	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	sul1, int1
Otu0662	Bacteria	Proteobacteria	Betaproteobacteria	Betaproteobacteria_unclassified	Betaproteobacteria_unclassified	Betaproteobacteria_unclassified	sul2, oxa-1
Otu0745	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Zoogloea	sul1, int1
Otu0869	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Novispirillum	sul1, int1
Otu1041	Bacteria	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	sul1, int1
Otu1540	Bacteria	Proteobacteria	Betaproteobacteria	Betaproteobacteria_unclassified	Betaproteobacteria_unclassified	Betaproteobacteria_unclassified	sul1, int1

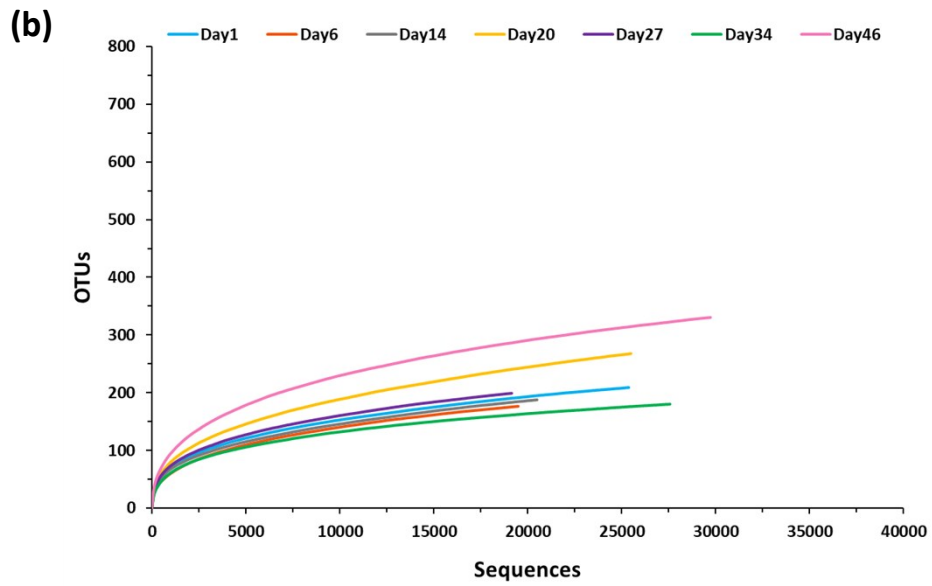
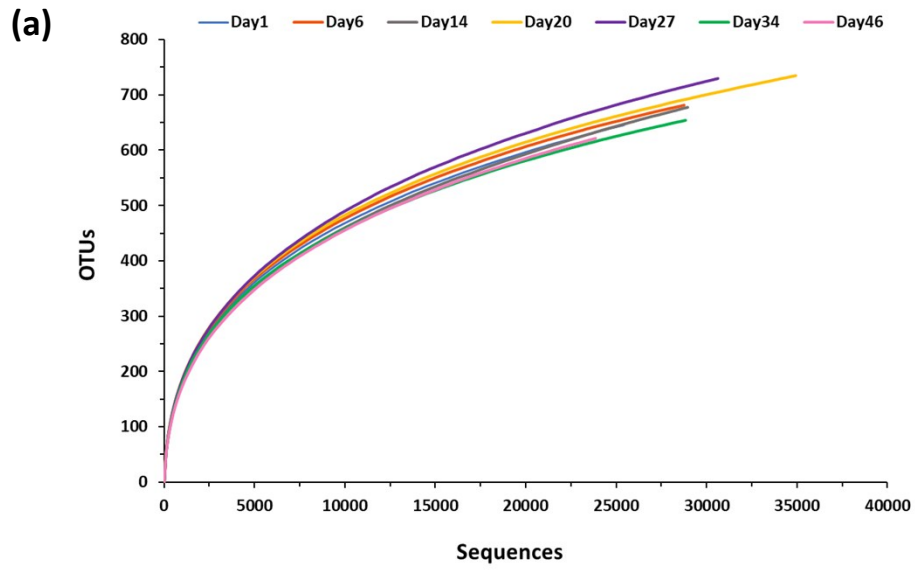
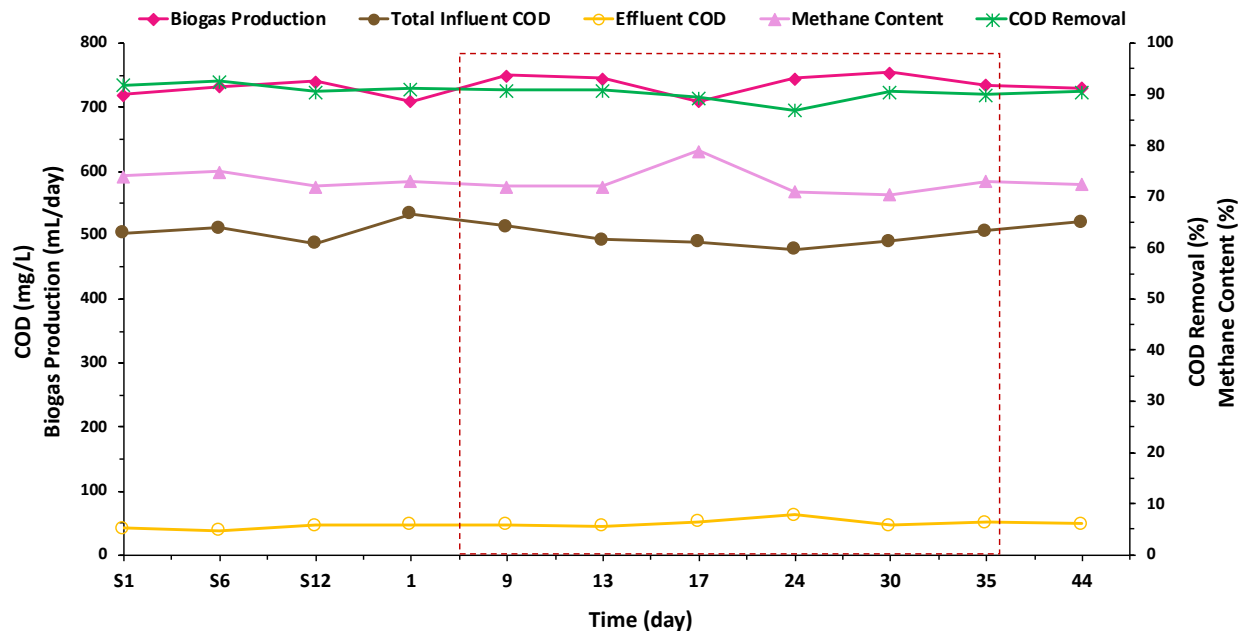
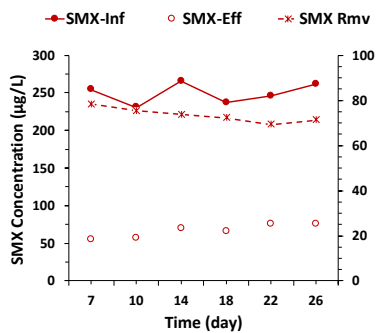


Figure S1. Rarefaction curves for (a) biomass and (b) effluent microbial community samples of the AnMBR.

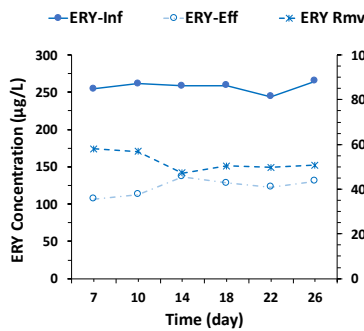
(a)



(b)



(c)



(d)

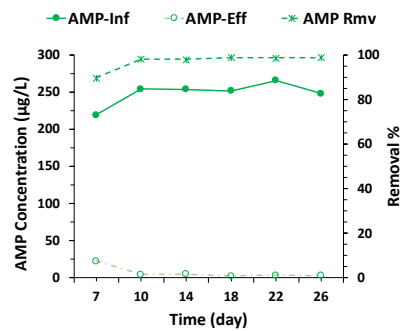


Figure S2. Performance of the AnMBR in (a) COD removal and biogas production, and (b) sulfamethoxazole, (c) erythromycin, and (d) ampicillin removal efficiency. In (a), days S1, S6, and S12 represent sampling points during confirmation of steady-state reactor performance. The AnMBR was operated for 48 days prior to the operational period depicted in the above figure. Additional reactor performance data, in which the reactor performed similarly, is provided in our previous paper.⁸

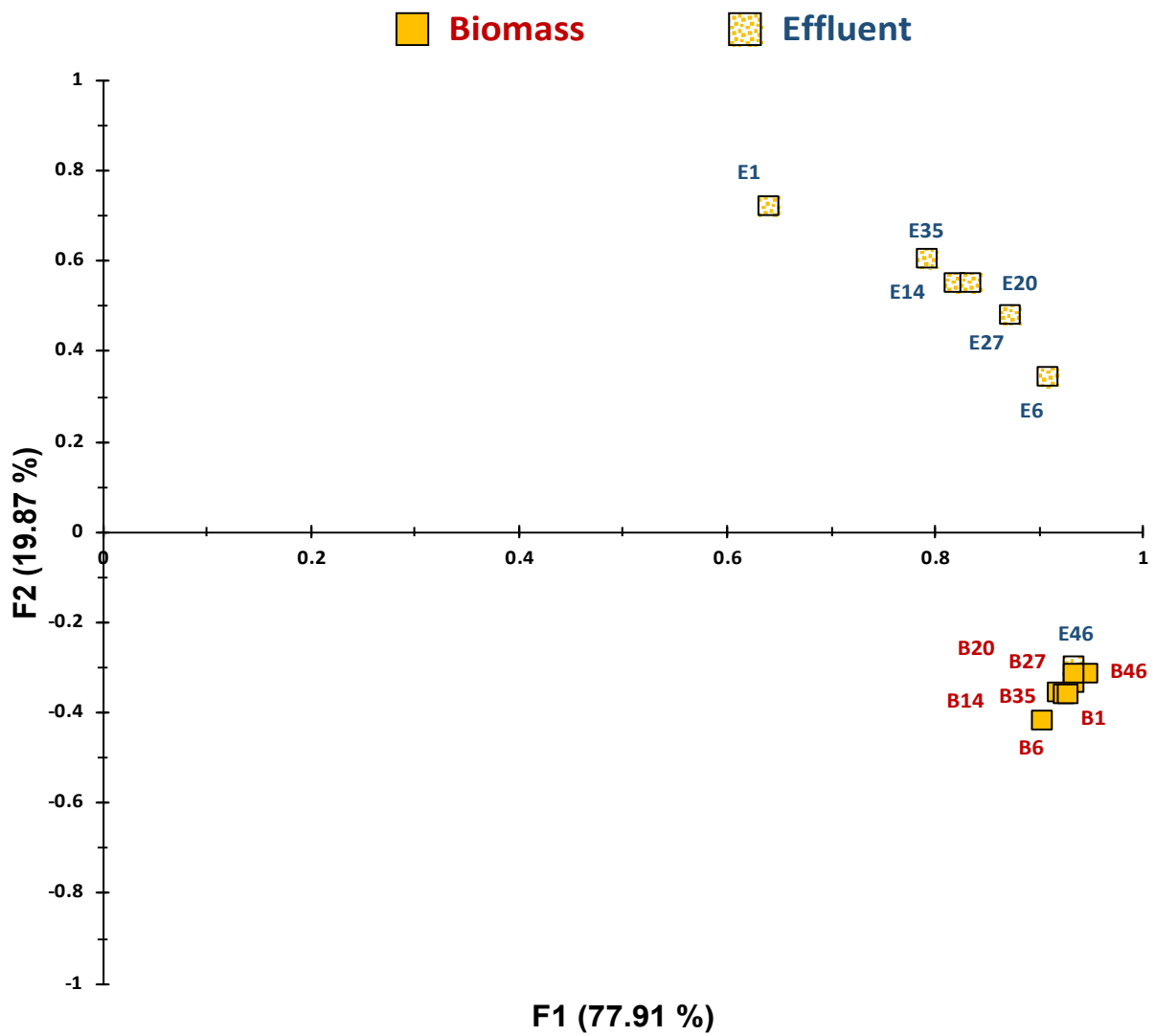
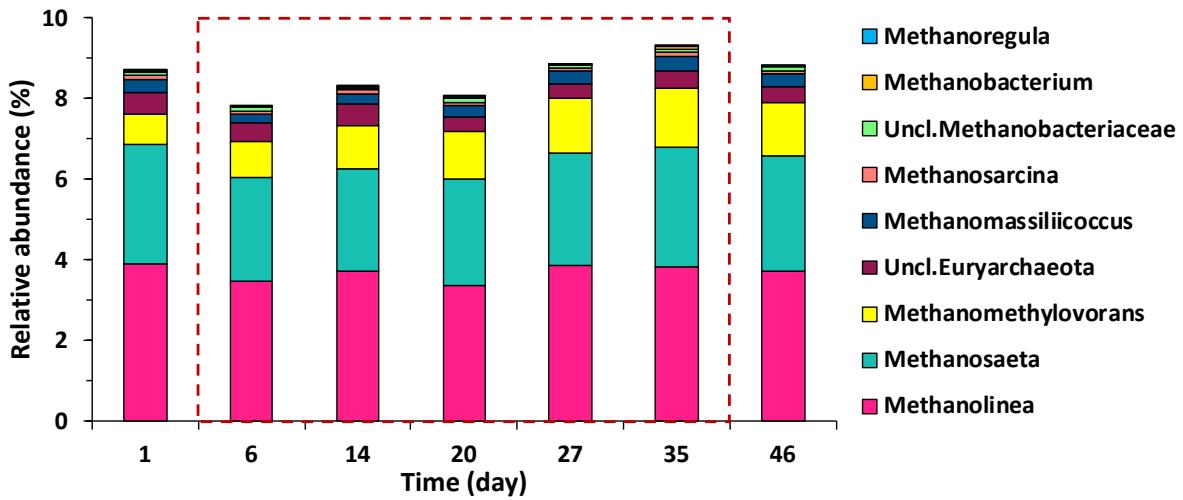


Figure S3. PCA of ARG profiles in the biomass and effluent of the AnMBR.

(a)



(b)

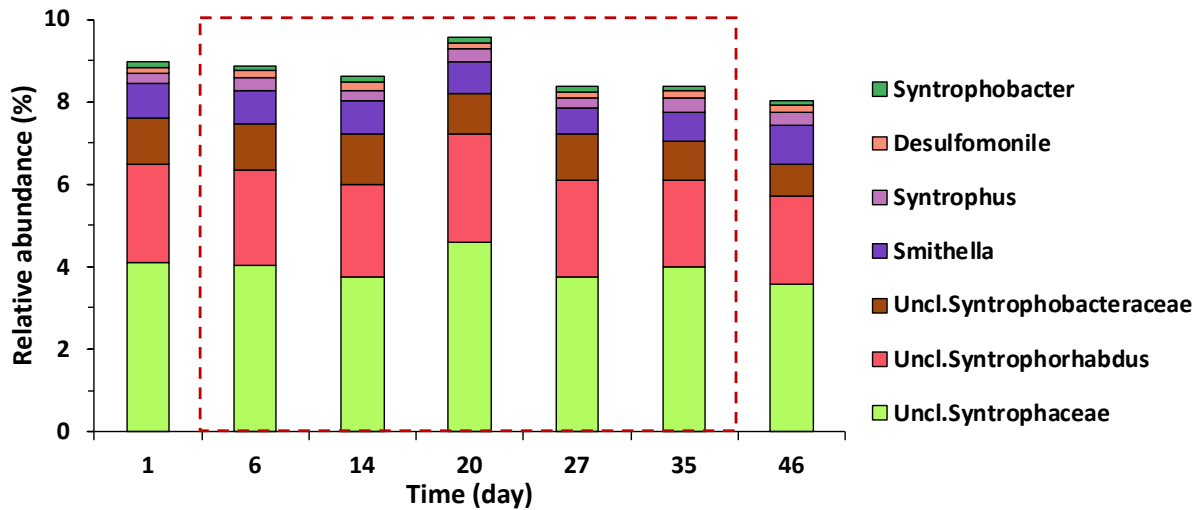


Figure S4. Relative abundance of (a) methanogens and (b) syntrophic bacteria in the biomass of the AnMBR throughout the experimental period. Day 1 represents pre-antibiotics period, days 6, 14, 20, 27 and 35 represent antibiotics loading period (area bordered by red dashed line) and day 46 represents post-antibiotics period.

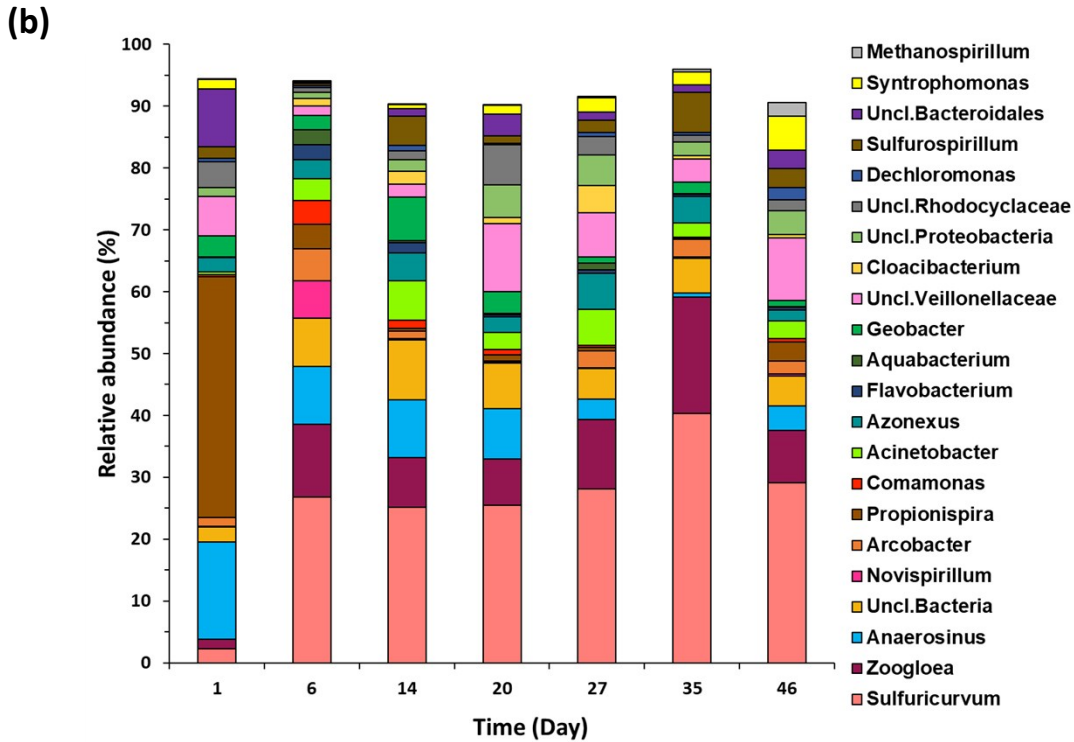
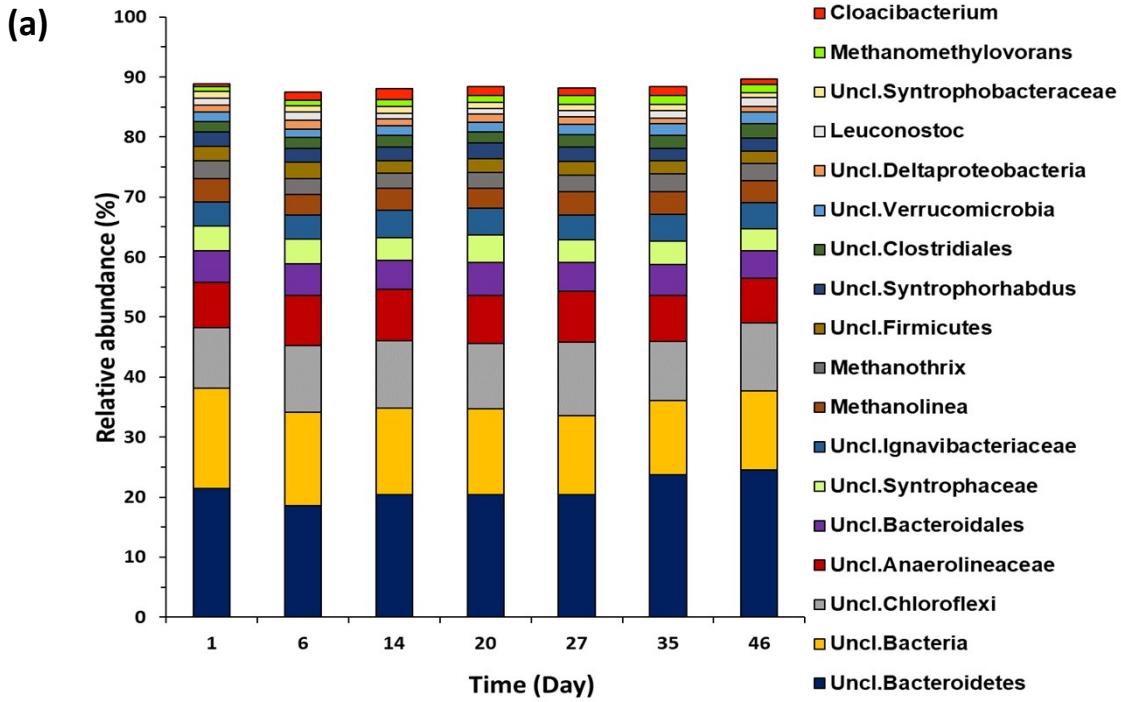


Figure S5. Relative abundance of the (a) Biomass and (b) effluent Microbial Community in genus Level throughout the experimental period. Day 1 represents pre-antibiotics period, days 6, 14, 20, 27 and 35 represent antibiotics loading period and day 46 represents post-antibiotics.

COD Mass Balance in the Reactor

Influent COD: 453 ± 32 mg/L

Effluent COD: 45 ± 10 mg/L

Mixed Liquor Volatile Suspended Solid: 9.6 ± 0.3 g/L

Biogas Methane Production: 536 ± 14 mL/d

Effluent Dissolved Methane: 112 ± 5 mL/d

Wasted Biomass: 10 mL/d

Volume of the Reactor: 5 L

gCOD/gVSS: 1.42^9

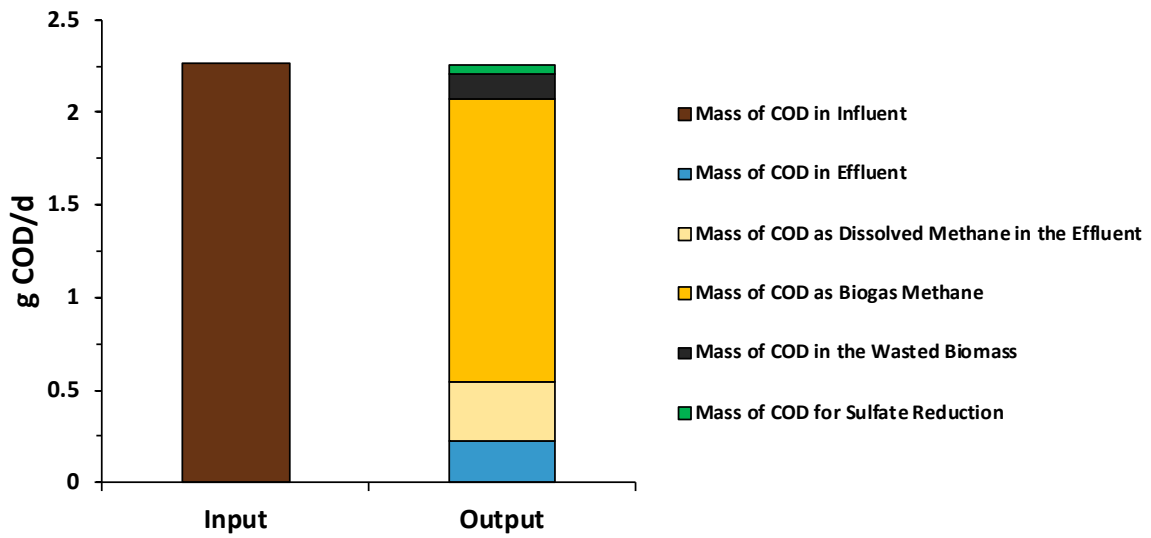


Figure S6. COD mass balance in the reactor.

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