Antibiotic Resistance Gene Dynamics in Anaerobic Digestion: Interactions with Prokaryotes and Viruses

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Text S1

Sampling methods. Food waste and digestate samples were collected from eight large-scale mesophilic food waste treatment plants, located in an eastern coastal province of China, between July and December 2019. Among these plants, one employed a dry-wet anaerobic digestion (AD) process, while the others utilized a wet AD process, involving a total of nine anaerobic digesters for food waste treatment. Sampling occurred after all bioreactors had been stably operating for over two years, and the reactors were reported to be functioning normally at the time. Before sampling, the food waste underwent pretreatment to remove impurities and separate oils. Digestate samples were collected from effluent pipelines near each bioreactor. Before collection, the sampling valve was opened for 5 minutes to flush the valve and pipeline. To ensure representativeness, samples were collected every 2 hours at three different time points. After sealing in sterile tubes, the samples were transported to the laboratory on dry ice within 24 hours. One portion was used for physicochemical measurements, while another portion was sealed in 50 mL sterile tubes and stored at -80°C for DNA extraction. The three replicate samples designated for metagenomic analysis were homogenized prior to DNA extraction.

Text S2

Physicochemical properties analysis. Upon returning to the laboratory, the collected food waste samples were immediately analyzed for protein, carbohydrate, ammonia nitrogen, and pH levels. If all analyses could not be completed on the same day, samples were stored at 4°C and subsequently analyzed as soon as possible. The pH was measured using a pH meter (model FE20, Mettler Toledo, Shanghai, China). Protein content was determined using the Lowry assay, with samples treated with Folin-Ciocalteu reagent and measured at a wavelength of 750 nm. Carbohydrate content was analyzed using the phenol-sulfuric acid method. Ammonia nitrogen levels were quantified using the Nessler's reagent colorimetric method. Chemical oxygen demand (COD) was measured using the dichromate method. The concentration of volatile fatty acids (VFAs) was detected using a GC-HPLC system (Agilent 1200, Agilent Technology, USA) equipped with a 7.8 × 300 Aminex HPX-87-H chromatographic column (Bio-Rad, USA).

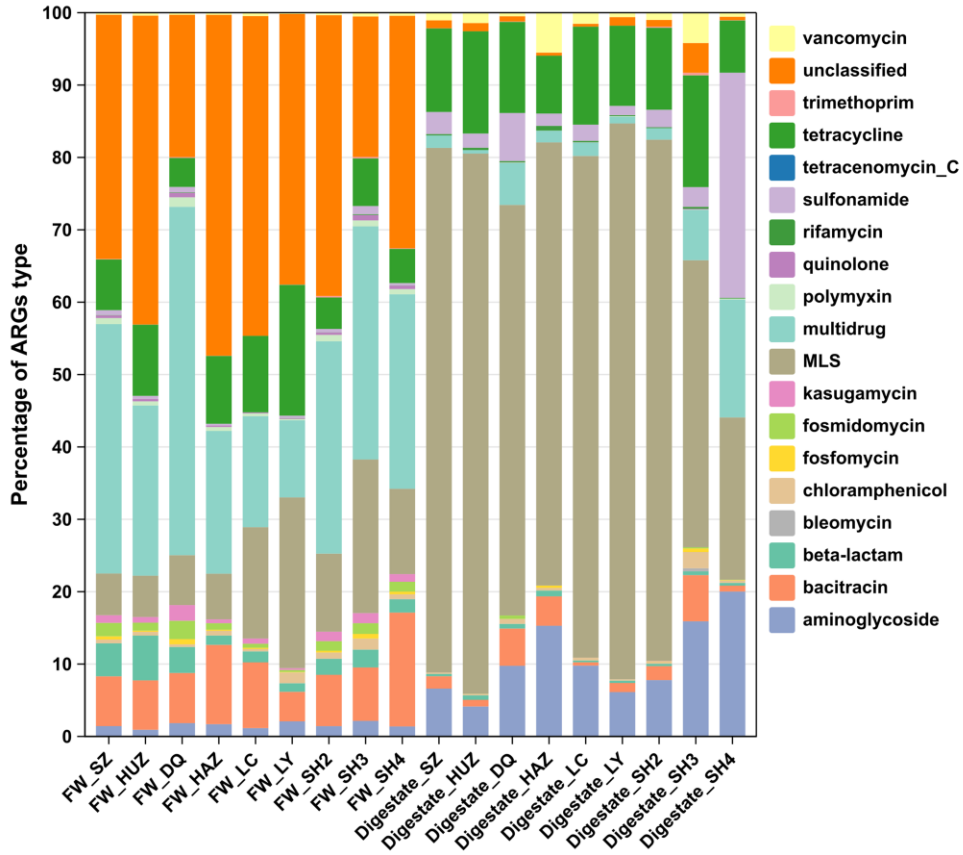

Fig. S1 ARG abundance ratio in FW and digestate

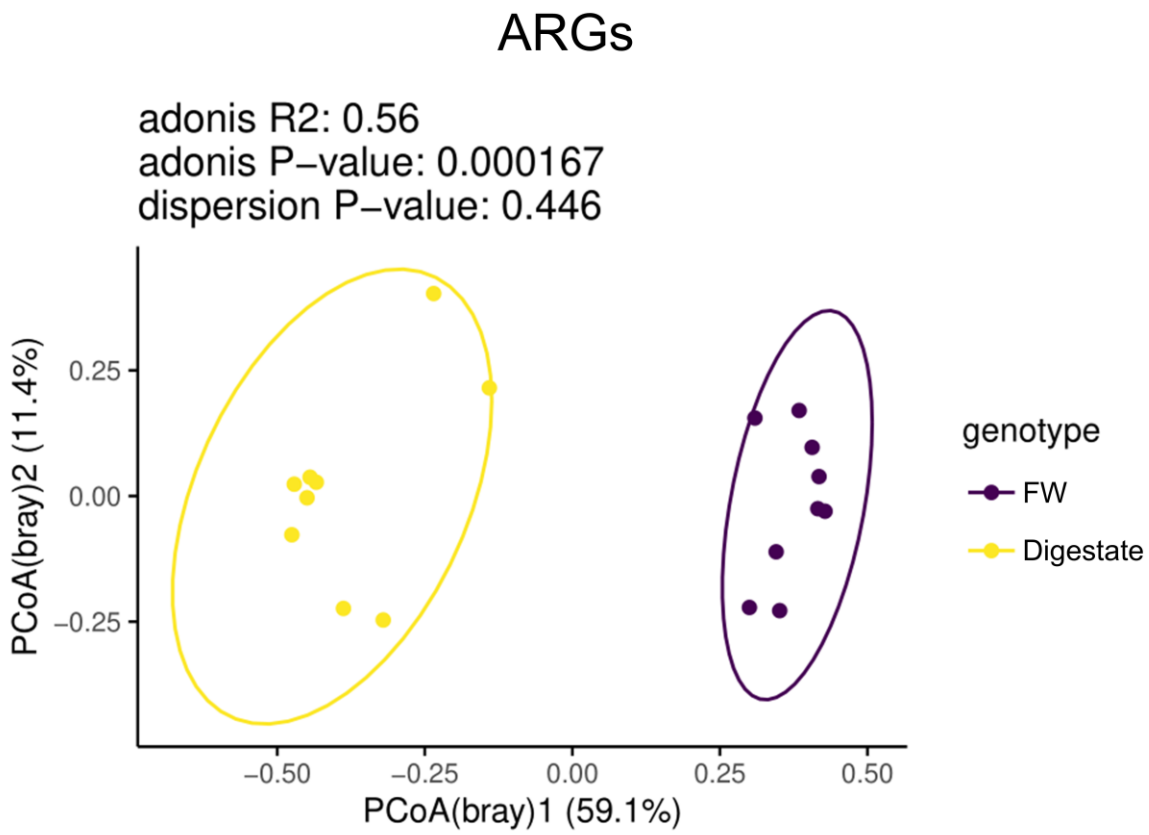

Fig. S2 PCoA plot of ARGs in FW and digestate

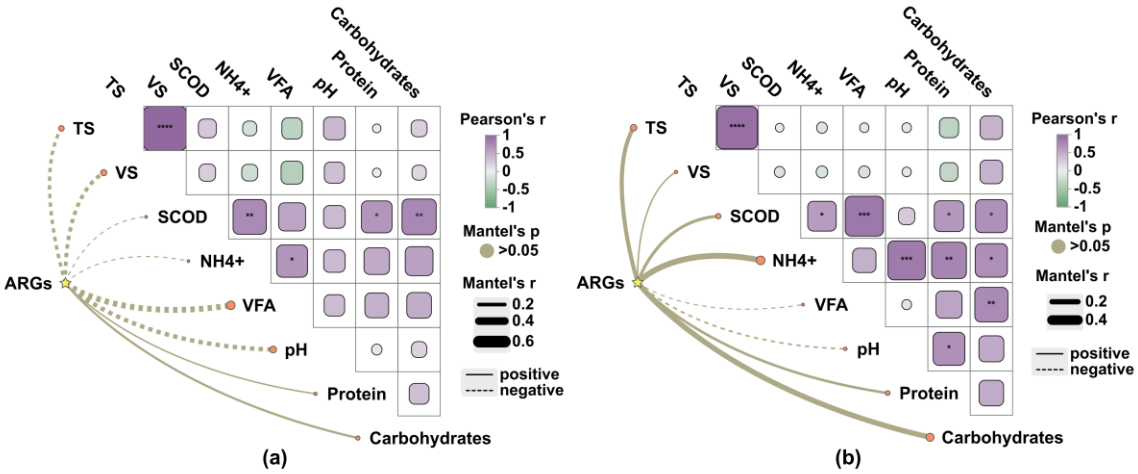

Fig. S3 Mantel test of ARGs and environmental factors in FW (a) and digestate (b)

Table S1 The detailed characteristics of food waste and digestate samples.

Sample name	Metagenomic Metadata Title in NGDC	Metagenomic Accession	Sampling site	Name of sampling site	TS (%)	VS (%)	SCOD (g/L)	Ammonia nitrogen	VFA (g/L)	pH	Protein (g/L)	Carbohydrates (g/L)	Substrate	AD process	Treatment scale (t/d)
FW_SZ	L1EEH070552_FWJ11_S90_L002	SAMC3098756	Jiangsu Suzhou	SZ	10.03	8.35	87.30	0.30	13.95	3.87	12.14	40.86	FW	CSTR	220
Digestate_SZ	L1EEH070568_FWJ12_S103_L002	SAMC3098757	Jiangsu Suzhou	SZ	2.75	1.29	17.80	3.07	4.95	7.83	4.75	0.57	Digestate	CSTR	
FW_HUZ	L1EEH070553_FWJ21_S91_L002	SAMC3098758	Zhejiang Huzhou	HUZ	5.25	3.56	96.90	1.16	26.02	4.81	5.77	47.93	FW	CSTR	320
Digestate_HUZ	L1EEH070569_FWJ22_S104_L002	SAMC3098759	Zhejiang Huzhou	HUZ	3.21	1.61	36.20	2.75	7.23	7.57	4.13	0.60	Digestate	CSTR	
FW_DQ	L1EEH070554_FWJ31_S92_L002	SAMC3098760	Zhejiang Deqing	DQ	6.84	5.68	57.20	0.27	12.71	4.17	7.99	23.54	FW	CSTR	100
Digestate_DQ	L1EEH070570_FWJ32_S105_L002	SAMC3098761	Zhejiang Deqing	DQ	5.59	3.34	1.90	1.18	0.55	7.71	0.21	0.13	Digestate	CSTR	
FW_HAZ	L1EEH070571_FWJ51_S106_L002	SAMC3098762	Zhejiang Hangzhou	HAZ	7.88	6.43	83.70	0.32	15.93	3.66	7.31	41.91	FW	CSTR	320
Digestate_HAZ	L1EEH070572_FWJ52_S107_L002	SAMC3098763	Zhejiang Hangzhou	HAZ	3.15	1.33	15.60	3.95	0.78	7.97	4.06	0.61	Digestate	CSTR	
FW_LC	L1EEH070573_FWJ71_S108_L002	SAMC3098764	Shandong Liaocheng	LC	9.85	7.85	78.30	0.38	8.41	3.74	9.32	46.82	FW	CSTR	220
Digestate_LC	L1EEH070574_FWJ72_S109_L002	SAMC3098765	Shandong Liaocheng	LC	2.51	0.89	8.90	3.61	0.49	8.02	2.45	0.32	Digestate	CSTR	
FW_LY	L1EEH070560_FWJ101_S95_L002	SAMC3098770	Shandong Linyi	LY	5.66	4.51	49.60	0.29	6.41	3.74	2.94	18.99	FW	CSTR	200
Digestate	L1EEH070561_FWJ1	SAMC3098771	Shandong	LY	1.89	0.81	9.00	1.86	5.37	7.37	1.12	0.13	Digestate	CSTR	

_LY	02_S96_L002		Linyi												
FW_SH2	L1EEH070562_FWJ1	SAMC3098772	Shanghai	SH2	9.60	7.45	105.40	1.06	30.95	4.54	17.36	51.89	FW	CSTR	600
	11_S97_L002														
Digestate	L1EEH070563_FWJ1	SAMC3098773	Shanghai	SH2	1.48	0.52	5.00	3.82	1.82	8.14	6.75	0.14	Digestate	CSTR	
_SH2	12_S98_L002														
FW_SH3	L1EEH070564_FWJ1	SAMC3098774	Shanghai	SH3	18.1	15.1	85.30	0.32	3.13	4.86	2.96	40.39	FW	Thöni	600
	21_S99_L002				2	0								TTV	
Digestate	L1EEH070565_FWJ1	SAMC3098775	Shanghai	SH3	6.39	3.10	39.90	6.08	10.59	8.23	6.37	10.61	Digestate	Thöni	
_SH3	22_S100_L002													TTV	
FW_SH4	L1EEH070566_FWJ1	SAMC3098776	Shanghai	SH4	6.72	5.38	80.30	0.72	9.81	3.68	9.44	46.22	FW	CSTR	500
	31_S101_L002														
Digestate	L1EEH070567_FWJ1	SAMC3098777	Shanghai	SH4	5.96	2.46	1.40	1.52	0.78	7.56	0.24	0.06	Digestate	CSTR	
_SH4	32_S102_L002														

Table S2 ARGs present in all 9 FW samples

ARG types	ARG subtyes
aminoglycoside	*aph(3'')-I, aph(6)-I, aadA, aadE, aph(3''')-III, aac(6')-I*
bacitracin	*bacA*
beta-lactam	*penA, class C beta-lactamase, OXA-212, OXA-211, ampC, OXA-12, OXA-309, PBP-1A, PBP-1B*
chloramphenicol	*chloramphenicol exporter, floR, catA*
fosmidomycin	*rosB, rosA*
kasugamycin	*kasugamycin resistance protein ksgA*
MLS	*lnuA, ermB, macA, macB, ermA, erm(TR), ermC, lmrP, mefA, lnuB, lsa*
multidrug	*multidrug_ABC_transporter, mtrE, EmrB-QacA family major facilitator transporter, cmeA, bicyclomycin-multidrug_efflux_protein_bcr, multidrug transporter, mdfA, mdtG, emrE, mexH, ceoB, emrD, mdtA, cmeB, mdtF, mexC, adeC, mdtE, emrK, mdtH, oprM, acrF, adeA, mdtC, mexD, mepA, mexY, mdtB, mdtK, mexI, oprN, acrA, mdtN, mexG, acrB, mdtD, TolC, mdtM, mexB, mdtP, smeD, smeB, adeB*
polymyxin	*arnA*
quinolone	*abaQ*
tetracycline	*tetV, tetL, tetZ, tet37, tetR, tet44, tetP, tetK, tetO, tet32, tet35, tetM, tet34, tet31*
vancomycin	*vanR, vanS*
unclassified	*transcriptional regulatory protein CpxR cpxR, antibiotic resistance rRNA adenine methyltransferase, tmrB, bacterial regulatory protein LuxR, two-component system_response regulator EvgA, mycinamicin-resistance protein myrB, DNA-binding transcriptional regulator gadX*

Table S3 ARGs present in all 9 digestate samples

ARG types	**ARG subtyes**
aminoglycoside	*aad(6), aadA, aadE, ant(9)-I, aph(3'')-III*
bacitracin	*bacA*
MLS	*erm(35), erm(TR), ermA, ermB, ermC, ermF, ermG, ermT, lnuB, lsa, macB, mefA, vatB*
multidrug	*EmrB-QacA family major facilitator transporter, mepA, mexY, mtrE*
rifamycin	*ADP-ribosylating transferase_arr*
sulfonamide	*sul1, sul2, sul3*
tetracycline	*tet31, tet34, tet35, tet43, tetL, tetM, tetO, tetP, tetS, tetV, tetW, tetX1, tetX5, tetZ*
unclassified	*tmrB, transcriptional regulatory protein CpxR cpxR*
vancomycin	*vanR, vanS*

Table S4 List of emerging ARGs in digestate after AD

ARG types	**ARG subtyes**
aminoglycoside	*aac(6')-30-aac(6')-Ib', aadB, aph(3'')-IV, aph(3')-VII*
beta-lactam	*CARB-7, OXA-118, OXA-142, OXA-205, OXA-21, OXA-226, OXA-3, OXA-34, OXA-37, OXA-380, OXA-4, OXA-53, OXA-85, VEB-2, VEB-3, VEB-8*
bleomycin	*bleO*
fosfomycin	*fosX*
MLS	*erm(33), oleD, vatD, vgaB*
tetracycline	*tet31*
trimethoprim	*dfrB1, dfrB2, dfrB6*
vancomycin	*vanB, vanW*