

Table S1 The physicochemical characteristics of dewatered waste activated sludge (WAS) and the inoculum sludge

parameter	inoculum sludge (55°C)	inoculum sludge (37°C)	dewatered WAS
TS (% wt)	8.23	8.51	18.67
VTS (% wt)	4.32	4.63	9.54
VFA (mg/L)	ND	ND	ND

ND: not detected

Table S2 Relative proportions and significance analysis of performance parameters in T_MP reactors across three operational stages*.

Parameter	Stage	Relative proportions (%)			P-value
		T_PE	T_PVC	T_PS	
Methane production	I	96.81	108.90	103.70	0.0829
	II	95.39	96.83	99.28	0.5975
	III	94.83	105.36	97.86	0.2329
	P-value	0.9171	0.0618	0.5264	—
VTS degradation rate	I	98.12	99.50	119.12	0.60
	II	101.12	96.54	101.79	0.49
	III	98.24	103.64	100.70	0.36
	P-value	0.5111	0.3212	0.3721	—
STOC	I	90.13	87.70	86.73	0.35
	II	101.71	99.38	105.18	0.84
	III	126.03	122.99	123.89	0.92
	P-value	0.0018	0.0006	0.008	—

*: Relative proportion indicates the ratio of performance parameters in the T_MP reactor compared to the control reactor.

Table S3 Relative proportions and significance analysis of performance parameters in M_MP reactors across three operational stages*.

Parameter	Stage	Relative proportions (%)			P-value
		M_PE	M_PVC	M_PS	
Methane production	I	68.80	70.73	60.09	0.2579
	II	66.48	59.60	53.52	0.0625
	III	41.83	30.37	33.87	0.0088
	P-value	6.39E-05	2.23E-06	8.78E-05	—
VTS degradation rate	I	124.04	98.71	104.90	0.2621
	II	110.12	123.19	96.32	0.3392
	III	88.21	90.64	79.04	0.1398
	P-value	0.3236	0.0951	0.0009	—
STOC	I	108.12	83.99	94.14	0.3014
	II	122.30	114.41	114.13	0.4283
	III	106.57	102.70	109.64	0.8616
	P-value	0.4366	0.1207	0.3533	—

*: Relative proportion indicates the ratio of performance parameters in the M_MP reactor compared to the control reactor.

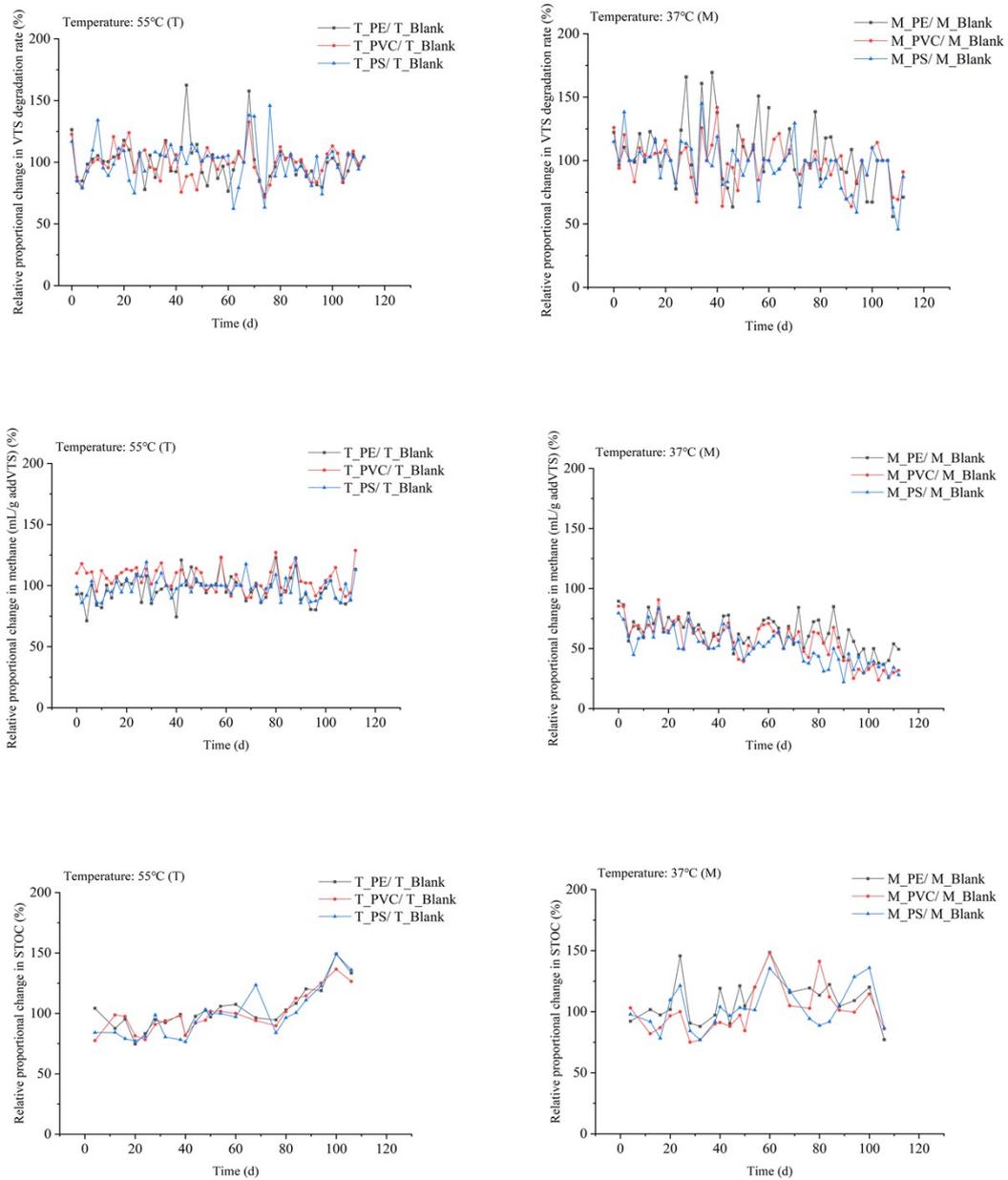


Fig. S1 Changes in relative proportions of VTS degradation rate, methane production, and STOC during different operational stages in thermophilic and mesophilic AD. The relative proportion indicates the ratio of performance parameters in the MP reactor compared to the control reactor.

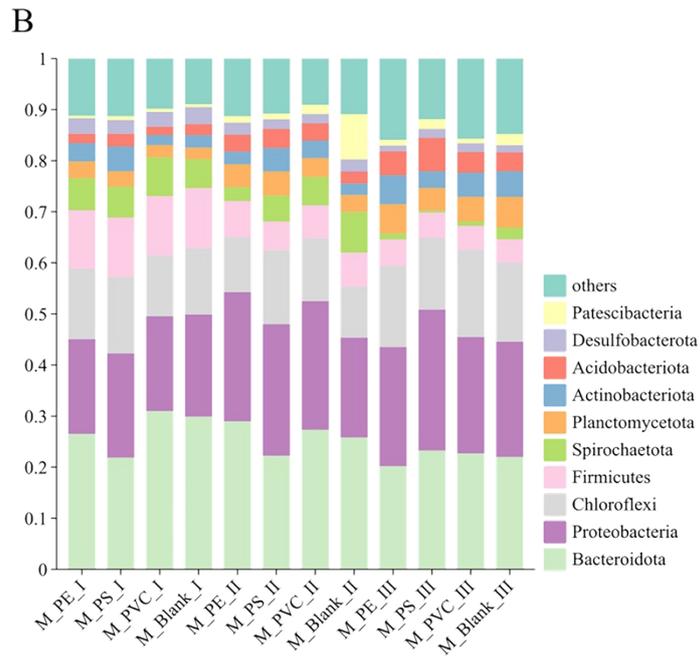
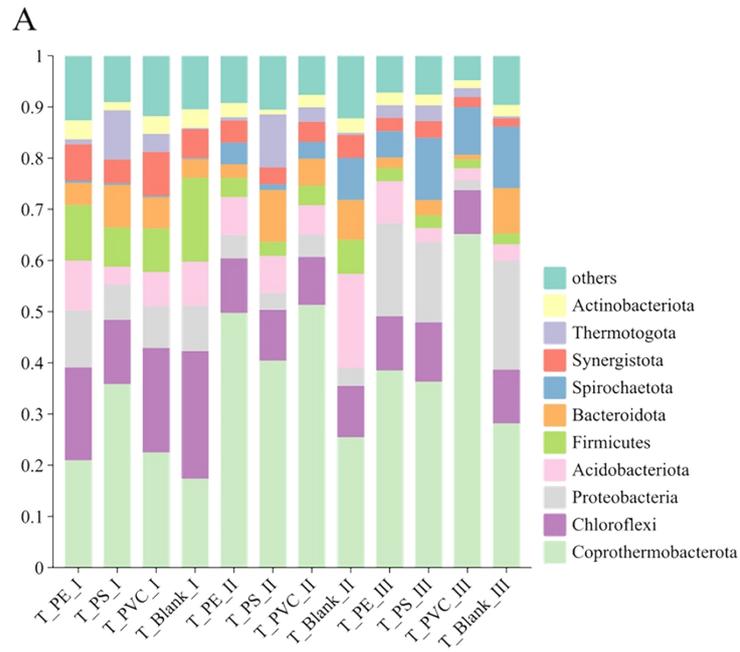
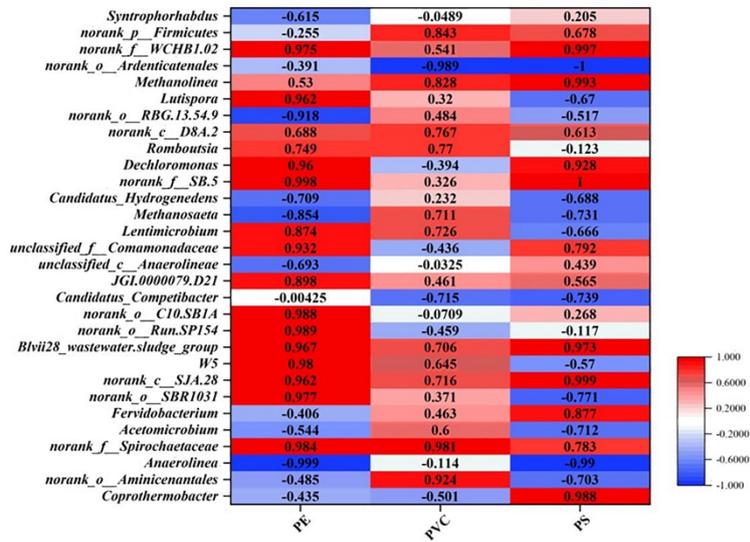


Fig. S2. Distribution of dominant phyla in microbial communities (A: thermophilic reactors; B: mesophilic reactors)

A



B

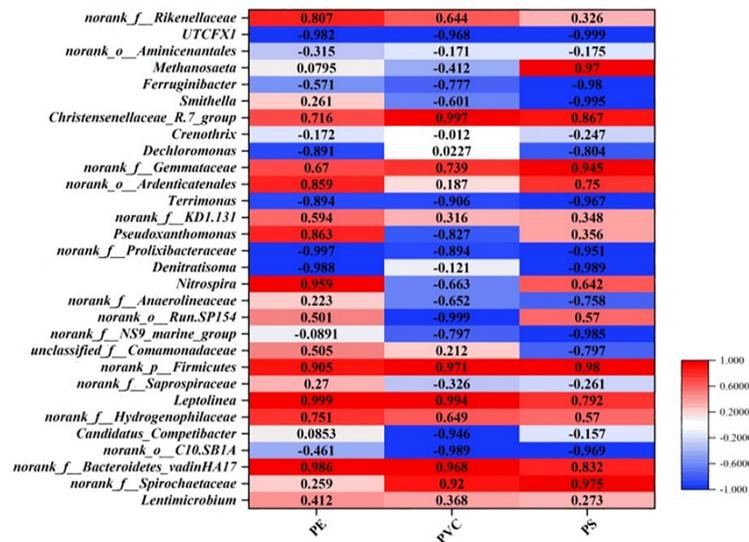


Fig. S3. Heatmap of Pearson correlation coefficient between dominant bacterial genus and methane production (A: thermophilic reactors; B: mesophilic reactors).

Table S4 Taxonomic annotation information of metagenome-assembled genomes (MAGs) in stage III of the mesophilic system (annotated via GTDB-Tk).

NAME	Domain	Phylum	Class	Order	Family	Genus	Species
bin02	Bacteria	Chloroflexota	Anaerolineae	UCB3	/	/	/
bin03	Bacteria	Gemmatimonadota	Gemmatimonadetes	Gemmatimonadales	GWC2-71-9	SPDF01	/
bin04	Bacteria	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	CADEFQ01	/
bin05	Bacteria	Actinobacteriota	Thermoleophilimia	Solirubrobacterales	Sep-70	RHKX01	RHKX01 sp018263295
bin06	Bacteria	Acidobacteriota	Aminicenantia	Aminicenantiales	UBA4085	UBA4085	/
bin07	Bacteria	Actinobacteriota	Actinomycetia	Nanopelagicales	UBA10799	JADKAV01	/
bin08	Bacteria	Desulfobacterota	Syntrophia	Syntrophales	Smithellaceae	UBA8904	/
bin09	Bacteria	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	RPQJ01	/
bin10	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	PHOS-HE28	PHOS-HE28	PHOS-HE28 sp016788405
bin11	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Thiobacillaceae	PFJX01	/
bin12	Bacteria	Bacteroidota	Bacteroidia	AKYH767-A	2013-40CM-41-45	CAINVI01	CAINVI01 sp016713765
bin13	Bacteria	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	RPQJ01	RPQJ01 sp018059685
bin14	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Thiobacillaceae	JACAED01	/
bin15	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	TH1-2	Terricaulis	/
bin16	Bacteria	Proteobacteria	Gammaproteobacteria	Competibacterales	Competibacteraceae	Competibacter	Competibacter phosphatis
bin17	Bacteria	Acidobacteriota	Blastocatellia	Pyrinomonadales	Pyrinomonadaceae	OLB17	OLB17 sp016789265
bin18	Bacteria	Nitrospirota	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira_A	Nitrospira_A sp003456605
bin19	Bacteria	Nitrospirota	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira_A	Nitrospira_A defluvii_A
bin20	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	ML635J-15	JAGOEF01	JAGOEF01 sp017997855
bin21	Bacteria	Acidobacteriota	Blastocatellia	RBC074	RBC074	/	/

Continued Table S4 Taxonomic annotation information of metagenome-assembled genomes (MAGs) in stage III of the mesophilic system (annotated via GTDB-Tk).

NAME	Domain	Phylum	Class	Order	Family	Genus	Species
bin22	Bacteria	Actinobacteriota	Acidimicrobia	Acidimicrobiales	Microtrichaceae	AWTP1-35	/
bin23	Bacteria	Caldisericotota	Caldisericia	JAAYUI01	JAAYUI01	/	/
bin24	Bacteria	Caldisericotota	Caldisericia	JAAYUI01	JAAYUI01	JAGNLA01	/
bin25	Bacteria	Chloroflexota	Anaerolineae	Anaerolineales	EnvOPS12	UBA12294	/
bin26	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	JJ008	JJ008 sp002069045
bin27	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	BACL12	/	/
bin28	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Ferruginibacter	Ferruginibacter sp018266715
bin29	Bacteria	Proteobacteria	Gammaproteobacteria	Methylococcales	Methylomonadaceae	KS41	/
bin30	Bacteria	Proteobacteria	Gammaproteobacteria	Competibacterales	Competibacteraceae	Competibacter_A	/
bin31	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Thiobacillaceae	PFJX01	/
bin32	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	M0108	/
bin33	Bacteria	Spirochaetota	Spirochaetia	Treponematales	UBA8932	VKGY01	/
bin34	Bacteria	Firmicutes_A	Clostridia	Oscillospirales	Acutalibacteraceae	UBA1447	UBA1447 sp002069695
bin35	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales	UBA8199	UBA8199	UBA8199 sp003527065
bin36	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	PHOS-HE28	PHOS-HE28	PHOS-HE28 sp018267155
bin37	Bacteria	Chloroflexota	Anaerolineae	SSC4	SSC4	SSC4	/
bin38	Bacteria	Chloroflexota	Anaerolineae	Promineofilales	Promineofilaceae	Promineofilum	/
bin39	Bacteria	Caldisericotota	Caldisericia	Cryosericales	Cryoseriaceae	Cryosericum	/
bin40	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	CAIWKO01	CAIWKO01	/

Note: '/' indicates unannotated taxonomic ranks.