Supporting Information

Anaerobic Co-digestion of Cattle Manure and Cucumber Residues: Biogas Production Potential and Microbial Community Characteristics

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In total, there are three texts, three figures and three tables, and the document length is ten pages.

Text S1 Raw Materials and Inoculum.

Fresh dairy manure was collected from the dairy barn of Yinxiang Weiye Group Co., Ltd. and thoroughly mixed using a blender (JYL-C63V, Joyoung Co., Ltd., China). The cucumber vines were collected from the Jinan Modern Agricultural Demonstration Park, then shredded to under 5 mm in size with a food waste grinder (DAOGRS MCD-56, DAOGRS INC., China). Each material was stored at 4°C in a fridge until further use.

The sludge was collected from the Hengyuan agricultural biogas project (using vegetable residues and swine manure as feedstock) in Changqing District, Jinan. The collected sludge was centrifuged at 2000 r/min for 15 min, and the sediment was employed as inoculum. Prior to use, the inoculum was stored in sealed containers within a 4°C walk-in cooler.

The chemical properties of raw materials and inoculants are shown in Table S1.

Text S2 Determination and Methodology of Gas Phase.

Gas composition analysis (including CO_2, CH_4, N_2, and O_2) was performed using a gas chromatograph with the thermal conductivity detector (TCD detector) (Beijing Spectrum Analysis Company, Beijing, China). Helium was used as the carrier gas at a flow rate of 5.2 mL/min. The temperature of the detector room was maintained at 200℃, while the initial temperature of the oven was 40℃ and then rapidly increased to 60℃ within 1 min. The amount of methane produced per gram of raw material expressed by mL CH_4/g VS and the amount of methane loaded into the reactor at start-up were corrected by subtracting the amount of methane produced per gram of inoculum from the amount of methane loaded into the control reactor.

Text S3 Statistical Analysis.

Due to the existence of interference data in the original sequencing data, the data were further filtered and spliced by RDP and QIIME2 software to obtain valid data, which were subjected to cluster analysis and species classification analysis with 97% consistency. Based on the obtained OTU results, species annotation was performed on each OUT by the BLAST online analysis tool to obtain the species information and species abundance data of the microbial communities of bacteria and archaea, respectively.

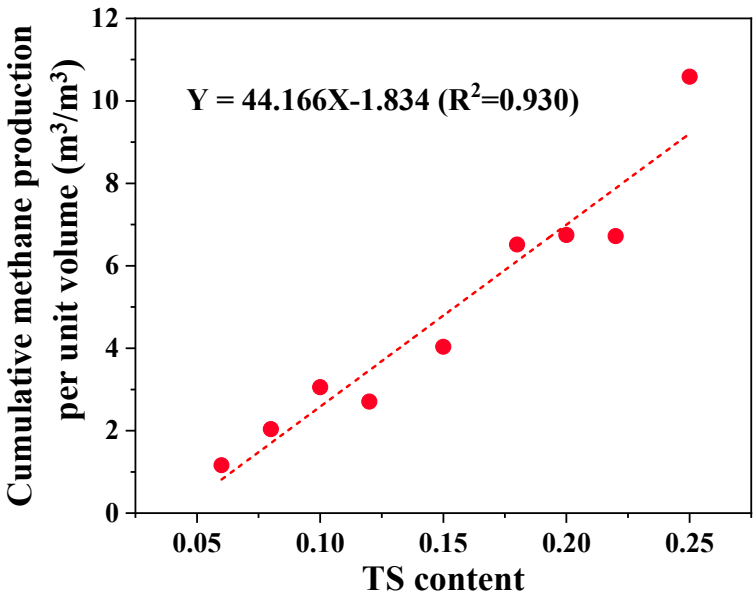

Figure S1 The linear relationship between TS content and volumetric cumulative methane production.

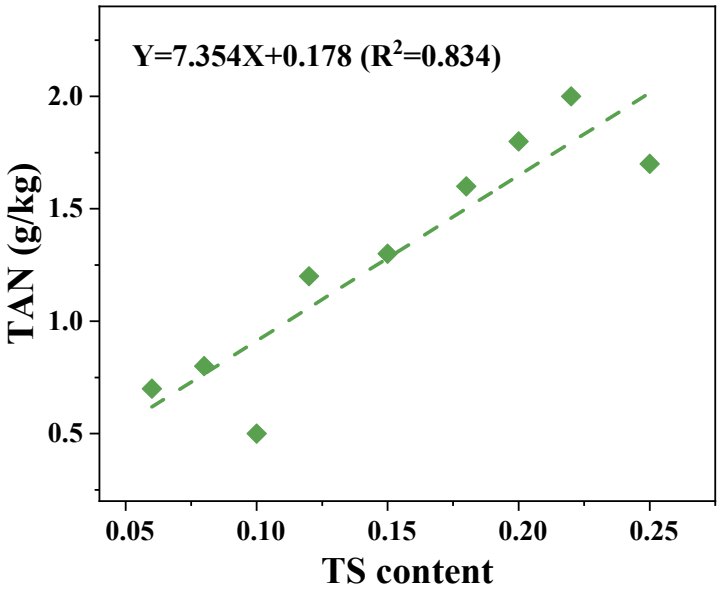

Figure S2 The linear relationship between TS content and TAN concentration.

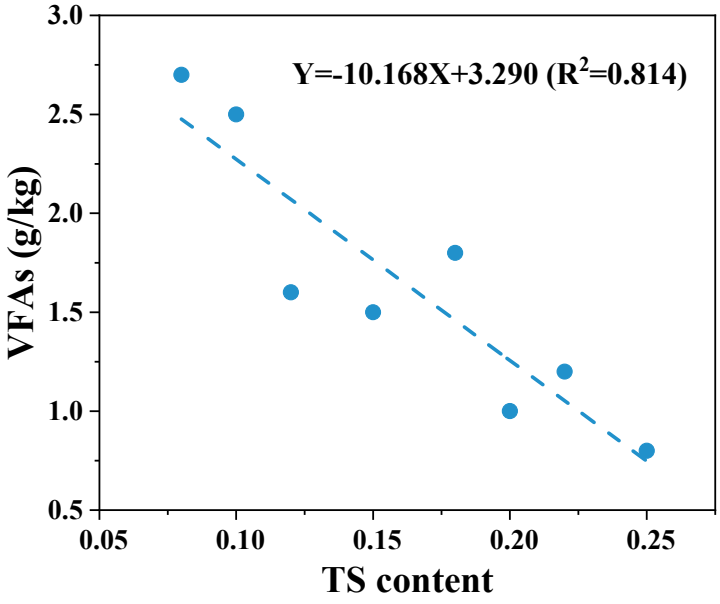

Figure S3 The linear relationship between TS content and VFAs concentration.

Table S1 Characteristics of feedstocks and inoculums for anaerobic co-digestion

Measurement Indicator	Cucumber Vines	Dairy Manure	Inoculum
Water Content (%)	81.4±0.02	62.2±0.4	62.6±0.8
Total Solid (%)	18.6±0.4	37.8±0.3	37.4±0.4
Volatile Solids (%)[b]	14.3±0.8	16.1±0.02	10.2±0.03
Volatile Solids/Total Solids (%)	76.6±0.7	42.6±0.2	27.3±0.04
pH	ND[a]	8.0±0.2	8.3±0.4
Total Carbon (%)[b]	47.0±0.01	45.4±0.0	16.7±0.02
Total Nitrogen (%)[b]	2.40±0.4	2.4±0.0	0.8±0.01
Carbon Nitrogen Ratio	19.4±0.2	18.5±0.0	21.9±0.02
Hemicellulose (%)[b]	25.2±0.5	25.1±0.7	14.3±0.2
Cellulose (%)[b]	27.4±0.7	23.4±0.5	6.3±0.5
Lignin (%)[b]	7.6±0.04	6.1±0.07	5.1±0.7

[a] ND = Not Detected. [b] Calculate according to dry weight, the rest according to wet weight.

Table S2 The mass of materials added for different treatments

Treatment	Cucumber Vines (g)	Dairy Manure (g)	Inoculum (g)	Water (g)	Total Mass (g)
H1	40.23	40.23	105.07	614.46	800
H2	53.64	53.64	140.10	552.62	800
H3	67.05	67.05	175.12	490.77	800
H4	80.46	80.46	210.15	428.92	800
H5	100.58	100.58	262.69	336.15	800
H6	120.70	120.70	315.22	243.38	800
H7	134.11	134.11	350.25	181.54	800
H8	147.52	147.52	385.27	119.69	800
H9	167.63	167.63	437.81	26.92	800

Table S3 Relative abundance of bacteria and archaea on the initial and peak period

Microbial community	Taxonomy	Treatment			
		H1-initial	H9-initial	H1-peak period	H9-peak period
Bacteria	*Firmicutes*	50.92(ab)	43.26(bc)	37.13(c)	57.27(a)
	Bacteroidetes	34.51(b)	34.09(b)	45.36(a)	34.11(b)
	Proteobacteria	4.34(b)	12.52(a)	1.86(b)	4.38(b)
	Spirochaetes	0.78(d)	2.14(c)	6.04(a)	5.04(b)
	Fibrobacteres	0.33(c)	0.95(b)	3.70(a)	0.44(c)
	Chloroflexi	1.79(a)	0.73(b)	0.68(b)	0.55(b)
	unidentified_Bacteria	1.86(a)	0.98(b)	0.81(bc)	0.53(c)
	Tenericutes	0.35(d)	1.48(a)	0.90(c)	1.19(b)
	Synergistetes	1.12(b)	0.89(c)	1.34(a)	0.79(d)
	Armatimonadetes	0.61(a)	0.24(b)	0.13(c)	0.05(d)
	Others	3.40(a)	2.72(b)	2.04(c)	1.65(d)
Archaea	*Methanoculleus*	9.04(c)	58.29(b)	58.42(b)	68.26(a)
	Methanobrevibacter	20.82(b)	24.05(a)	2.49(d)	11.30(c)
	Methanosarcina	15.34(b)	4.05(d)	21.09(a)	8.80(c)
	Methanospirillum	7.60(a)	4.46(b)	3.78(c)	2.63(d)
	Methanobacterium	2.69(a)	0.98(b)	0.75(b)	0.94(b)
	Methanosaeta	0.74(a)	0.37(b)	0.40(b)	0.19(c)
	Methanocorpusculum	0.30(b)	0.42(a)	0.05(d)	0.12(c)
	Methanosphaera	0.30(a)	0.15(b)	0.01(c)	0.07(bc)
	Methanogenium	0.26(a)	0.21(a)	0.02(b)	0.06(b)
	Methanimicrococcus	0.07(b)	0.17(a)	0.02(b)	0.06(b)
	Others	42.82(a)	6.91(c)	12.94(b)	7.57(c)

Note: Different superscript letters (a, b, c, d) within the same row indicate statistically significant differences ($p < 0.05$) among experimental groups based on ANOVA and Tukey's HSD test.