

Supporting Information

Figures:

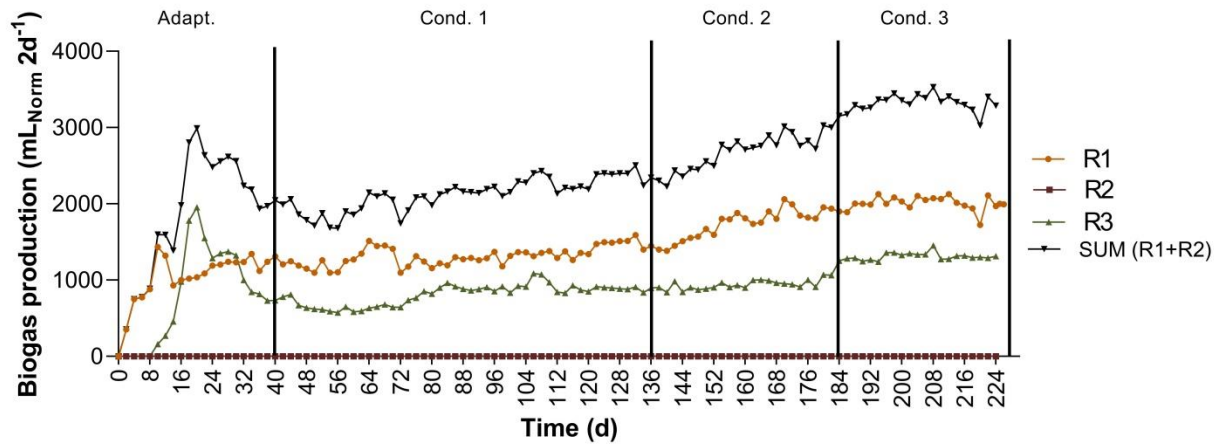


Figure S1. Biogas production in R1, R3 and sum of R1 and R3 during the 224 days of reactor operation.

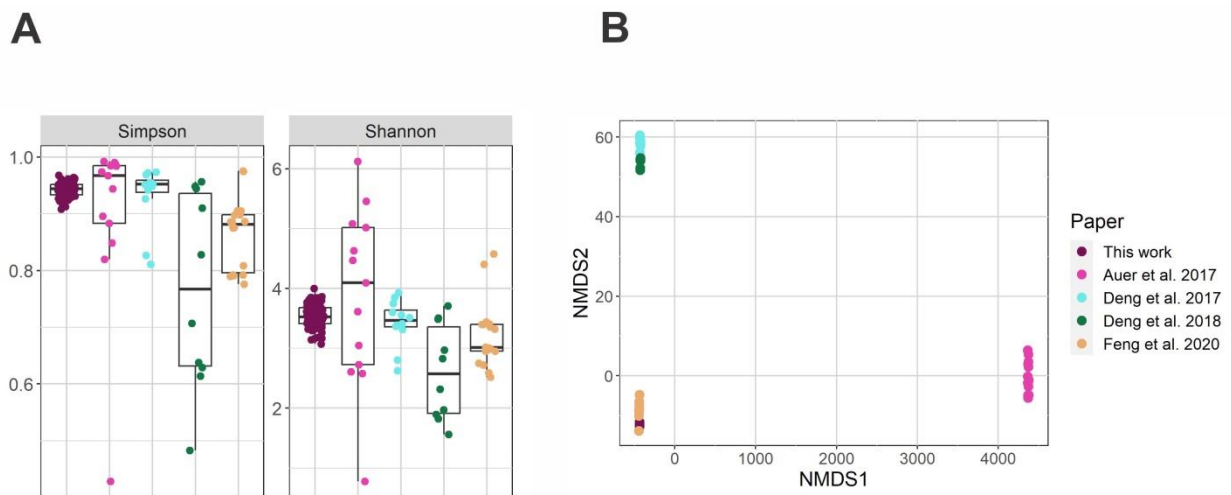


Figure S2. Bacterial community dynamics and structure comparing this work with other four anaerobic digestion systems. (A) Simpson and Shannon diversity indices; (B) The non-metric

multidimensional scaling (NMDS) based on Bray–Curtis dissimilarity index showing the relationships among different anaerobic digestion systems.

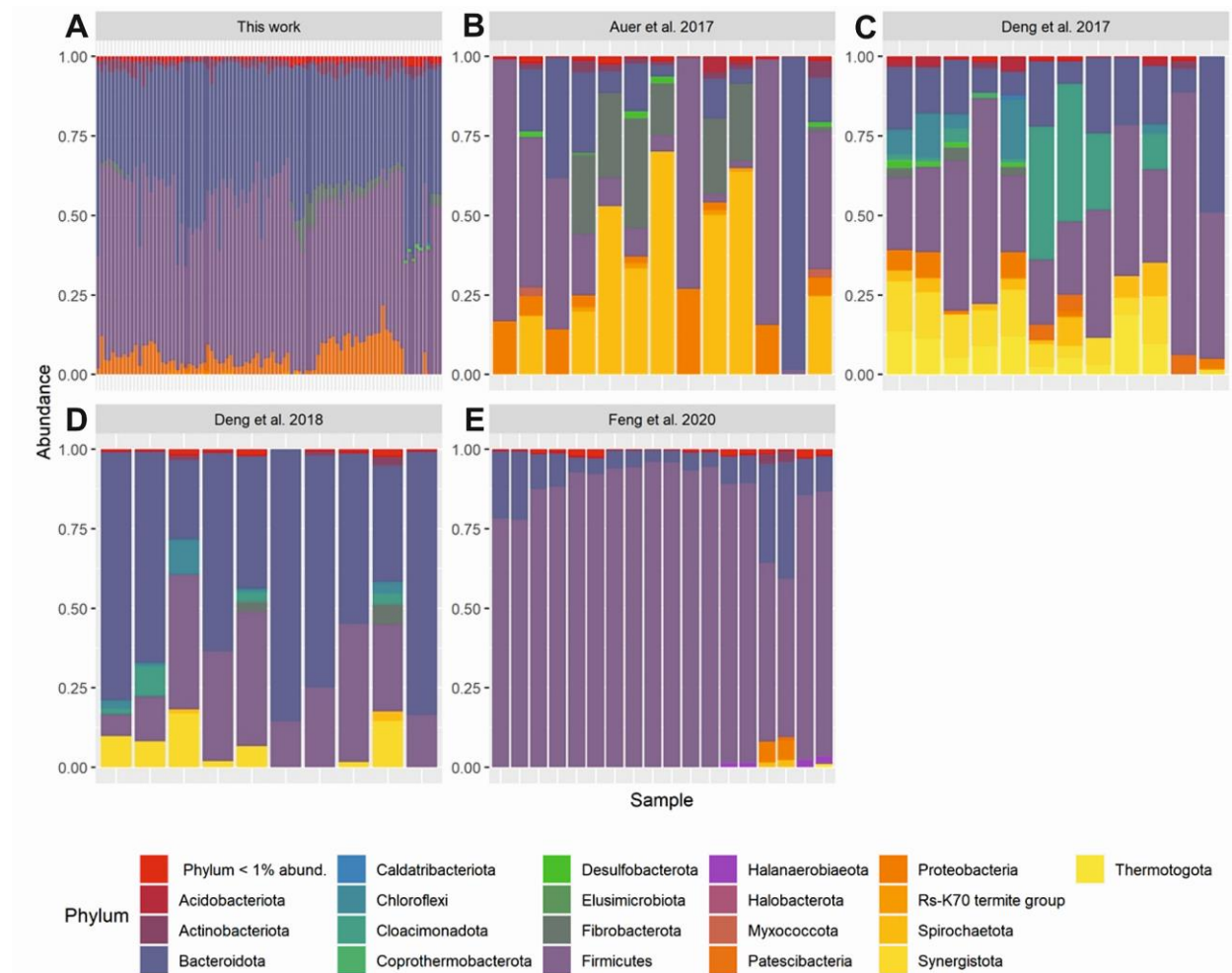


Figure S3. Relative abundance of the bacterial community at phylum level; (A) This work; (B) Auer et al. 2017 [1]; (C) Deng et al. 2017 [2]; (D) Deng et al. 2018 [3]; (E) Feng et al. 2020 [4].

Tables:

Table S1. Average of the physical-chemical parameters measured during the reactor operation for each reactor during the adaptation phase and the three different conditions applied to the system.

	R1				R2				R3				Total R1+R2+R3			
	Adapt.	Cond.1	Cond.2	Cond.3	Adapt.	Cond.1	Cond.2	Cond.3	Adapt.	Cond.1	Cond.2	Cond.3	Adapt.	Cond.1	Cond.2	Cond.3
Specific CH ₄ production (mL _{norm} g ⁻¹ VS)	51	63	56	49	0	0	0	0	94	85	70	52	145	148	126	101
Specific biogas production (mL _{norm} g ⁻¹ VS)	108	131	115	99	0	0	0	0	169	145	125	96	277	276	240	195
Biogas production (mL _{norm} 2d ⁻¹)	1105	1332	1749	2004	0	0	0	0	850	413	484	657	1955	1745	2233	2661
CH ₄ (%)	44	48	48	50	11	20	28	48	41	59	56	54				
O ₂ (%)	2	1	1	1	8	3	4	1	1	1	1	1				
CO ₂ (%)	34	42	44	44	14	10	4	22	22	29	31	35				
H ₂ (%)	0	0	0	0	0	0	0	0	0	0	0	0				
N ₂ (%)	21	9	7	6	67	67	65	29	35	11	12	11				
pH	7	7	8	8	9	9	9	8	8	8	8	8				
Ethanol (mg L ⁻¹)	5	4	5	1	12	5	6	1	3	1	0	0				
Lactate (mg L ⁻¹)	0	12	0	1	0	19	137	81	0	13	6	5				
Acetate (mg L ⁻¹)	325	338	141	194	1323	1835	1730	1193	189	69	82	98				
Propionate (mg L ⁻¹)	774	218	97	85	1038	748	537	463	56	13	0	12				
iso-Butyrate (mg L ⁻¹)	7	4	0	0	34	25	0	0	0	0	0	0				
n-Butyrate (mg L ⁻¹)	19	4	0	24	116	41	13	18	18	57	4	3				
iso-Valerate (mg L ⁻¹)	7	1	0	5	39	19	12	31	4	0	1	3				
n-Valerate (mg L ⁻¹)	7	1	0	5	12	7	2	6	0	0	0	2				
Total VFAs production (mg L ⁻¹)	1139	566	239	312	2462	2699	2437	1794	270	153	92	121	3871	3418	2768	2228
Volatile solids (% _{VS})	70	73	80	80	55	65	72	75	36	53	61	69				
Volatile solids fed (g _{VS} d ⁻¹)	5	5	8	10	3	3	5	7	2	3	4	7				
Total volatile solids degradation (%)	66 (±18.1) 44 (±7.5) 45 (±4.1) 28 (±9.1)															

Table S2. Experimental conditions of this study and in the studies used as comparison.

	Inoculum	Feedstock	Reactor type	Working volume	Feeding regime	Temperature	Goal
Current study	Beetle larvae gut microbiome	Wheat straw	3 stirred tanks connected in series	5L	Semi- continuous	Mesophilic	Carboxylate and methane production
Auer et al. 2017	Termite gut microbiome	Wheat straw	Stirred tank	0.4 L	Batch	Mesophilic	Carboxylate production (methane inhibited with BES)
Deng et al. 2017	Ruminal microbiota + anaerobic sludge from kitchen waste	Rice straw from a paddy field	AMPTS, Bioprocess Control, Lund, Sweden and serum bottles	0.25L/0.08L	Batch	Mesophilic	Carboxylate and methane production using different inoculum ratios
Deng et al. 2018	Ruminal microbiota + anaerobic sludge from kitchen waste	Mixture of corn stalks, guinea grass, and rice straw	-	2.5L	Semi- continuous	Mesophilic	Carboxylate and methane production using different organic loads
Feng et al. 2020	Cattle manure + thermophilic inoculum from pilot-scale reactor (running with cattle manure as the main feedstock)	Different ratios of cover crop silage, co-ensiled cover crop and straw with or without addition of cattle manure	Stirred tank	15L	Semi- continuous	Thermophilic	Methane production during different feeding compositions

References

1. Auer, L.; Lazuka, A.; Sillam-dussès, D.; Miambi, E.; Donohue, M. O.; Hernandez-raquet, G. Uncovering the Potential of Termite Gut Microbiome for Lignocellulose Bioconversion in Anaerobic Batch Bioreactors. *Front. Microbiol.* **2017**, *8* (December), 1–14. <https://doi.org/10.3389/fmicb.2017.02623>.
2. Deng, Y.; Huang, Z.; Ruan, W.; Zhao, M. Co-Inoculation of Cellulolytic Rumen Bacteria with Methanogenic Sludge to Enhance Methanogenesis of Rice Straw. *Int. Biodeterior. Biodegradation* **2017**, *117*, 224–235. <https://doi.org/10.1016/j.ibiod.2017.01.017>.
3. Deng, Y.; Huang, Z.; Ruan, W.; Miao, H.; Shi, W.; Zhao, M. Enriching Ruminal Polysaccharide-Degrading Consortia via Co-Inoculation with Methanogenic Sludge and Microbial Mechanisms of Acidification across Lignocellulose Loading Gradients. *Appl. Microbiol. Biotechnol.* **2018**, *102* (8), 3819–3830. <https://doi.org/10.1007/s00253-018-8877-9>.
4. Feng, L.; Perschke, Y. M. L.; Fontaine, D.; Nikolausz, M.; Ward, A. J.; da Rocha, U. N.; Corrêa, F. B.; Eriksen, J.; Sørensen, P.; Møller, H. B. Anaerobic Digestion of Co-Ensiled Cover Crop and Barley Straw: Effect of Co-Ensiling Ratios, Manure Addition and Impact on Microbial Community Structure. *Ind. Crops Prod.* **2020**, *144*. <https://doi.org/10.1016/j.indcrop.2019.112025>.