Supporting Information

Graphene addition to digestion of thin stillage can alleviate acidic shock and improve biomethane production

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Materials and Methods

Microbial profiling

The DNA extraction and further processing were performed by Shanghai Majorbio Bio-pharm Technology Co., Ltd to analyse the bacterial and archaeal communities (Shanghai, China).[1] DNA was extracted from sludge samples using E.Z.N.A.® Soil DNA Kit (Omega Bio-Tec, USA) following the manufacturer's protocol. The extracted DNA concentration was quantified by a spectrophotometer (Thermo Scientific, USA) whilst DNA integrity was checked by 1% agarose gel electrophoresis. For bacteria, the V3–V4 hypervariable regions were targeted to amplify the 16 rRNA gene with primers 338F (5'–ACTCCTACGGGAGGCAGCAG–3') and 806R (5'–GGACTACHVGGGTWTCTAAT–3') by polymerase chain reaction (PCR). For archaea, the V4 hypervariable regions of the 16S rRNA gene were amplified by PCR using primers 524F10extF (5'–TGYCAGCCGCCGCGGTAA–3') and Arch958RmodR (5'–YCCGGCGTTGAVTCCAATT–3'). All PCR reactions for each sample were conducted in triplicate. Afterwards, the triplicate resulting PCR products were well mixed, checked, purified, and quantified. Purified amplicons were pooled together in equimolar quantities and sequenced on an Illumina MiSeq platform (Illumina, USA). Operational taxonomic units (OTUs) clustering at a similarity of over 97% was performed using UPARSE 7.0 (http://drive5.com/uparse/). Meanwhile, taxonomic classification of each sequence was characterized by RDP Classifier 2.11 (https://sourceforge.net/projects/rdp-classifier/) against Silva database Release 132 (https://www.arb-silva.de/).

Biogas composition analysis

Biogas composition was assessed using a gas chromatograph system (Agilent 7890B, USA) equipped with a thermal conductivity detector and a 5A column (detailed analytical procedure was presented in Supplementary Material). The temperature of inject port and thermal conductivity detector was 150 °C and 300 °C, respectively. The column temperature was initially set at 50°C for 5 min, then increased to 200 °C at a heating rate of 30 °C/min. Argon was used as the carrier gas.

Soluble COD concentrations of pyrochar and graphene

To measure the soluble COD of pyrochar and graphene, oven dried pyrochar or graphene was added into the corresponding volume of distilled water to achieve 1 g pyrochar/L, 10 g pyrochar/L and 1 g graphene/L, respectively. Before measuring, the suspension samples were ultrasonically treated for 30 mins and then centrifuged at 10,000 rpm for 10 mins.

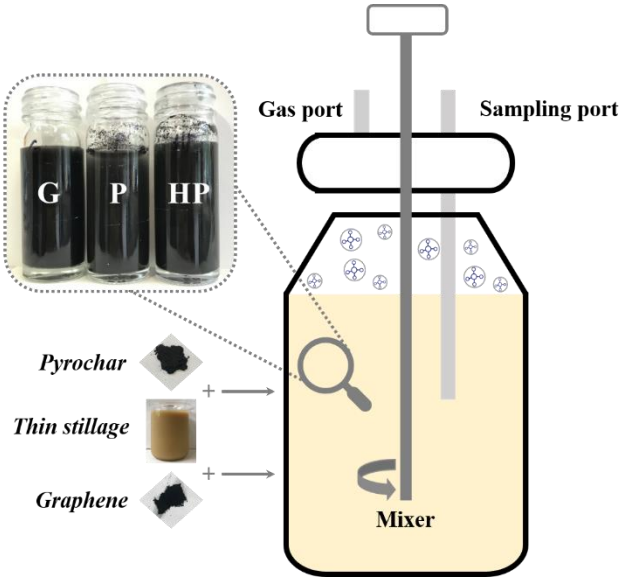

Figure S1. Schematic diagram of digesters for anaerobic digestion of thin stillage with conductive materials amendment (G: 1 g graphene/L suspension; P: 1 g pyrochar/L suspension; HP: 10 g pyrochar/L suspension).

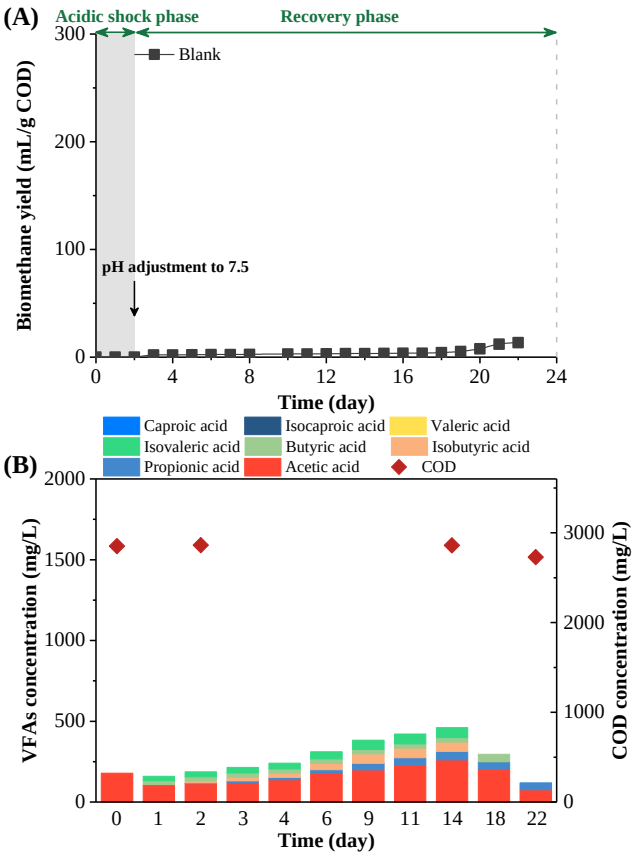

Figure S2. The performance of (A) biomethane yield, (B) VFAs formation and COD concentration of the blank group during anaerobic digestion after acidic shock.

Table S1 Characteristics of thin stillage and inoculum.

Parameter [a]	Thin stillage	Inoculum
TS (g/L)	36.60 ± 1.08	42.50 ± 0.42
VS (g/L)	33.13 ± 1.17	26.95 ± 0.21
pH	4.01	8.07
C (% TS)	44.65 ± 0.15	/
H (% TS)	5.73 ± 0.06	/
N (% TS)	5.13 ± 0.08	/
O (% TS)	44.48 ± 0.26	/
TN (mg/L)	1169.61 ± 8.44	/
NH_3-N (mg/L)	26.23 ± 0.70	/
TCOD (g/L)	51.13 ± 1.63	/
SCOD (g/L)	32.90 ± 1.14	/
SCarbohydrate (g/L)	11.69 ± 0.01	/

[a] TS = total solid; VS = volatile solid; TN = total nitrogen; NH_3-N = free ammonia; TCOD = total COD; SCOD= soluble COD; SCarbohydrate = soluble carbohydrate

Table S2 Soluble COD concentrations of pyrochar and graphene.

Material	Dosages (g/L)	Soluble COD concentrations (mg/L)
Pyrochar	1	95.25 ± 15.20
	10	88.45 ± 17.75
Graphene	1	46.55 ± 6.01

References:

1. Wang, X.; Sun, G.; Feng, T.; Zhang, J.; Huang, X.; Wang, T.; Xie, Z.; Chu, X.; Yang, J.; Wang, H.; Chang, S.; Gong, Y.; Ruan, L.; Zhang, G.; Yan, S.; Lian, W.; Du, C.; Yang, D.; Zhang, Q.; Lin, F.; Liu, J.; Zhang, H.; Ge, C.; Xiao, S.; Ding, J.; Geng, M., Sodium oligomannate

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