energies

Effects of Two-Stage Operation on Stability and Efficiency in Co-Digestion of Food Waste and Waste Activated Sludge

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Supplementary Information S1: Explanations for acetate and butyrate fluctuations affected by pH and operational situations in HR.

As shown in Figure 3, the concentration of acetate and butyrate was similar in most cases at Operation 1, 3 and 4, whereas the acetate prevailed over butyrate at Operation 2, which might be caused by the changes of metabolic pathways at different HRT. At the operation time of 1 d, 53 d and 79 d, the butyrate suddenly increased and maintained at a high level for 6-12 d before back to normal, and the decline of acetate was also observed at the same time. During the operation of H_2-reactor, the pH dropped to 4.8 and 4.5 at 1 d and 53 d respectively, and the reactor was broken at 79 d, which were assumed to be reasons for butyrate and acetate variation. The conversion of butyrate to acetate occurs according to the anaerobic oxidation reaction [1], as shown in Equation (1):

$$CH_3CH_2CH_2COO^- + 2H_2O \leftrightharpoons 2CH_3COO^- + H^+ + 2H_2 \quad \Delta G = +48.1 \qquad (1)$$

Where ΔG is the Gibbs free energy and a positive ΔG means the reversible reaction is thermodynamic favorable in standard condition. When the pH declined, the $[H^+]$ concentration increased and the reverse reaction of Eq. (1) was readily to occur, which results in the conversion from acetate to butyrate and also the reduction of hydrogen production.

Reference

1. Ding, H.B.; Liu, X.Y.; Stabnikova, O.; Wang, J.Y., Effect of protein on biohydrogen production from starch of food waste. *Water Sci. Technol.* 2008, 57, 1031–1036.

Table S1: Results of ANOVA test for comparison of the performance between MR1 and MR2.

In some cases, the homogeneity of variance assumptions were unsatisfied, the results from parametric test was not reliable, so the Mann-Whitney U test for independent samples was performed to determine if there were significant differences in the operation performance between MR1 and MR2.

Table S1. Results of ANOVA test for comparison of the performance between MR1 and MR2.

Parameters	Operation 1		Operation 2		Operation 3		Operation 4	
CH_4 production rate	*n*=22 *p*=0.143>0.05	Insig[1]	*n*=6 *p*=0.000<0.05	Sig[2]	*n*=11 *p*=0.000<0.05	Sig	*n*=17 *p*=0.000<0.05	Sig
CH_4 yield	*n*=22 *p*=0.09>0.05	Insig	*n*=6 *p*=0.000<0.05	Sig	*n*=11 *p*=0.000<0.05	Sig	*n*=17 *p*=0.000<0.05	Sig
CH_4 content	*n*=22 *p*=0.000<0.05	Sig	*n*=34 *p*=0.000<0.05	Sig	*n*=12 *p*=0.000<0.05	Sig	*n*=16 *p*=0.000<0.05	Sig
CO_2 content	*n*=22 *p*=0.000<0.05	Sig	*n*=34 *p*=0.000<0.05	Sig	*n*=12 *p*=0.000<0.05	Sig	*n*=16 *p*=0.000<0.05	Sig
pH	*n*=23 *p*=0.000<0.05	Sig	*n*=36 *p*=0.000<0.05	Sig	*n*=25 *p*=0.001<0.05	Sig	*n*=11 *p*=0.001<0.05	Sig
TVFA	*n*=26 *p*=0.506>0.05[a]	Insig	*n*=33 *p*=0.02<0.05[a]	Sig	*n*=25 *p*=0.000<0.05[a]	Sig	*n*=12 *p*=0.000<0.05[a]	Sig
NH_4^+-N	*n*=5 *p*=0.000<0.05	Sig	*n*=7 *p*=0.000<0.05	Sig	*n*=6 *p*=0.000<0.05	Sig	*n*=5 *p*=0.000<0.05	Sig
Alkalinity	*n*=7 *p*=0.000<0.05	Sig	*n*=12 *p*=0.000<0.05	Sig	*n*=16 *p*=0.004<0.05	Sig	*n*=11 *p*=0.407>0.05	Insig
VS	*n*=18 *p*=0.000<0.05	Sig	*n*=31 *p*=0.000<0.05	Sig	*n*=22 *p*=0.000<0.05	Sig	*n*=16 *p*=0.000<0.05	Sig
Total COD	*n*=8 *p*=0.07>0.05	Insig	*n*=7 *p*=0.5>0.05	Insig	*n*=6 *p*=0.015<0.05	Sig	*n*=6 *p*=0.03<0.05	Sig
Soluble COD	*n*=7 *p*=0.24>0.05	Insig	*n*=7 *p*=0.04<0.05	Sig	*n*=5 *p*=0.01<0.05	Sig	*n*=4 *p*=0.00<0.05	Sig
Total carbohydrate	*n*=7 *p*=0.92>0.05	Insig	*n*=9 *p*=0.7>0.05	Insig	*n*=6 *p*=0.014<0.05	Sig	*n*=4 *p*=0.029<0.05	Sig
Soluble carbohydrate	*n*=8 *p*=0.01<0.05	Sig	*n*=9 *p*=0.229>0.05	Insig	*n*=5 *p*=0.354>0.05	Insig	*n*=4 *p*=0.074>0.05	Insig
Soluble protein	*n*=8 *p*=0.354>0.05	Insig	*n*=8 *p*=0.01<0.05	Sig	*n*=6 *p*=1.0>0.05[a]	Insig	*n*=4 *p*=0.01<0.05	Sig

[a]: Mann-Whitney U test;

[1]: Insig means performance of MR1 and MR2 were not statistically significantly different.

[2]: Sig means there were significant difference in the performance between MR1 and MR2.

Figure S1: Taxonomic classification and the relative abundances of the major phyla in the bacterial 16S rRNA clone library of thermophilic hydrogen reactor

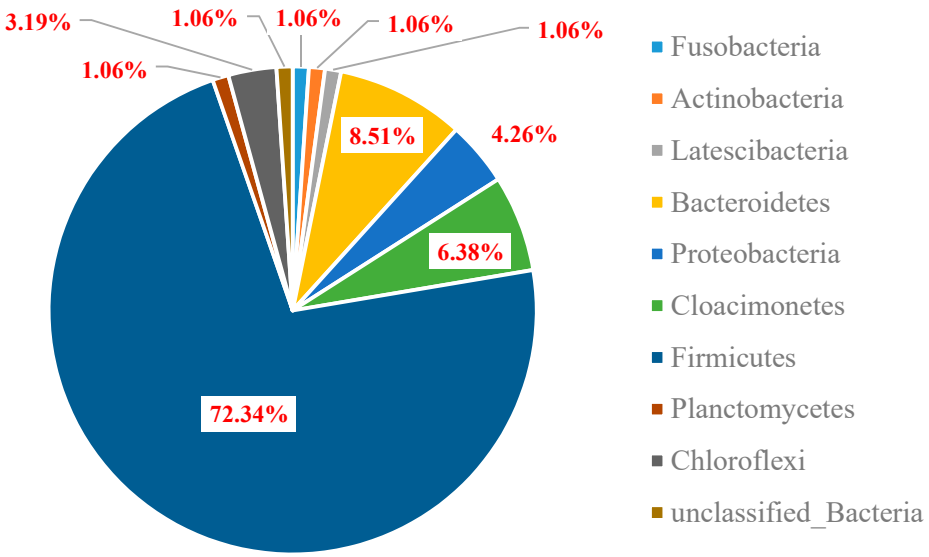

Figure S1. Taxonomic classification and the relative abundances of the 94 quality-checked clone sequences obtained in thermophilic HR with phylum as basis. The other 2 sequence with the length of less than 800 bp were filtered out before classification. All of the sequences were assigned to the domain *Bacteria*.

Table S2: Taxonomic classification and the relative abundances of the archaeal sequences by phyla and classes in MR1 and MR2.

Table S2. Taxonomic classification and the relative abundances of the archaeal sequences by phyla and classes in MR1 and MR2.

Phylum	Class	MR1	MR2
Euryarchaeota	*Methanomicrobia*	94.0%	19.5%
Euryarchaeota	*Methanobacteria*	0.0%	75.9%
Euryarchaeota	*Thermoplasmata*	1.2%	0.0%
Woesearchaeota		2.4%	0.0%
Thaumarchaeota		2.4%	3.4%