Optimization of Temperature-separated Two-stage Anaerobic Fermentation Process Treating waste activated sludge and food waste

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ABSTRACT

In this study, a temperature-separated two-stage anaerobic fermentation process of waste activated sludge (WAS) and food waste (FW) was performed to optimize the sludge retention time of methane producing stage (M-SRT) and sludge return ratios of the system (SRR). Results show that higher M-SRT improved the removal of organics and energy yield of the system but reduced the stability of hydrogen production in H_2-reactor. In this study, all the M-SRT conditions were below the threshold level. The highest total energy yield and volatile solid (VS) removal efficiency of the two-stage system of 8.98 kJ/g-VS and 63.8% was observed under the optimized M-SRT of 12 d. Return sludge could complement alkalinity for acidogenic stage especially with higher SRR, but the methanogens in it inhibited hydrogen production and caused stage transfer eventually. The optimized SRR was 1:1 with comprehensive consideration of energy yield, VS removal efficiency and alkali dosage. The dominant bacterial families in acidogenic stage were *Ruminococcaceae* and *Clostridiaceae* in this study.

KEYWORDS

Two-stage anaerobic fermentation; waste activated sludge; food waste; hydrogen production; methane production.

1. INTRODUCTION

Anaerobic fermentation can realize the waste reduction and bioenergy production from organic solid wastes[1]. In recent years, co-digestion has been widely used to improve reactor performance[2]. Meanwhile, the amount of food waste (FW) generated in 2015 was estimated to 56.57 million tons in mainland China[3]. However, traditional solid wastes disposal methods cannot efficiently transform the valuable energy in WAS and FW biomass. Compared with the single stage system, two-stage anaerobic fermentation could improve the energy recovery efficiency and stability of the system[4]. In this study, a temperature-separated two-stage anaerobic fermentation process for the treatment of waste activated sludge (WAS) and FW was performed to optimize the sludge retention time of methane producing stage (M-SRT) and sludge return ratios of the system (SRR).

2.MATERIALS AND METHODS

2.1 Seed sludge and substrates

The hydrogen-producing and methane-producing seed sludge were both obtained from the anaerobic digestion reactors with mixed substrates of WAS and FW. The hydrogen-producing seed sludge was base treated for 24 h, and then cultured for 7 days at 55℃, under anaerobic condition with FW as substrates. During base treatment, pH value of the sludge was adjusted to 12.0 with 1.0 mol/L NaOH, and then adjusted to 7.0 with 1.0 mol/L HCl after 24 h[5].

Substrates in this study included WAS and FW. Based on previous research work[6], the volatile solids (VS) of the substrates was 43.5g/L, among which the FW accounted for 54% (VS). The WAS was collected from the secondary sedimentation tank of Zhangguizhuang wastewater treatment plant in Tianjin, China. The FW was collected from the cafeteria of Tianjin University. The characteristics of substrates were shown in Table 1.

Table 1
Characteristics of waste activated sludge and food waste in feedstock.

Parameters	Waste activated sludge	Food waste	Feedstock
pH	7.1	5.5	6.6
VS/TS (%)	59.1	97.7	75.8
Total COD (mg/g-TS)	1009.7	1189.9	1080.0
Soluble carbohydrate (mg/g-TS)	0.4	289.8	146.7
Soluble protein (mg/g-TS)	0.2	18.8	10.2
Alkalinity (mg-$CaCO_3$/g-TS)	8.1	0.2	3.8

2.2 Experimental conditions

Schematic of the temperature-separated two-stage anaerobic fermentation process (BIOTECH-5BG, Shanghai Baoxing Bio-engineering Equipment Co. Ltd, China) was shown in Fig.1. The hydrogen producing stage (H_2-reactor)

was thermophilic fermentation with operating temperature of 55℃, while the methane producing stage (CH_4-reactor) was mesophilic fermentation with operating temperature of 37℃. The effective volume of two reactors were 1.5 L and 5 L, with fermentation broth volume of 1 L and 4 L respectively. Both the two reactors were stirred completely with stirring speed of 70 rpm and 300 rpm respectively. During the start-up period, the hydrogen-producing seed sludge and mixed substrates with volume ratio of 2:1 were added into H_2-reactor, and only seed sludge was added into CH_4-reactor. Both the two reactors were purged by nitrogen gas for 10 min before sealing. Before formal operation, mixed substrates were added into the reactors for continuous acclimation for a month. The pH value of the two reactors were controlled at 5.0-5.5 and 6.7-7.4 respectively by the addition of K_2HPO_4. The two reactors were fed twice a day.

The operation process for optimizing M-SRT was divided into three periods, with different M-SRT of 4 d, 8 d and 12 d. VS of the feedstock substrates in H_2-reactor was constant at 43.5g/L in each period. SRT and organic loading rate (OLR) was determined by changing feedstock volume of CH_4-reactor. Sludge from CH_4-reactor was recirculated into H_2-reactor to complement its alkalinity. The SRR (volume ratio of return sludge from CH_4-reactor to mixed substrates in H_2-reactor) under condition C-1 and C-2 was 1:1. Considering the insufficient sludge from CH_4-reactor under condition C-3, all the sludge was recirculated and the SRR was 2:3. The operation process for optimizing SRR was divided into three periods with different SRR at 1:1, 1:2 and 2:1. Total feedstock volume, SRT of hydrogen producing stage (H-SRT) and total VS of the mixed substrates in feedstock were constant in each period. SRR were determined by changing volume ratio of return sludge to mixed substrates. The actual SRT, OLR and operating time of different periods considering return sludge were shown in Table 2.

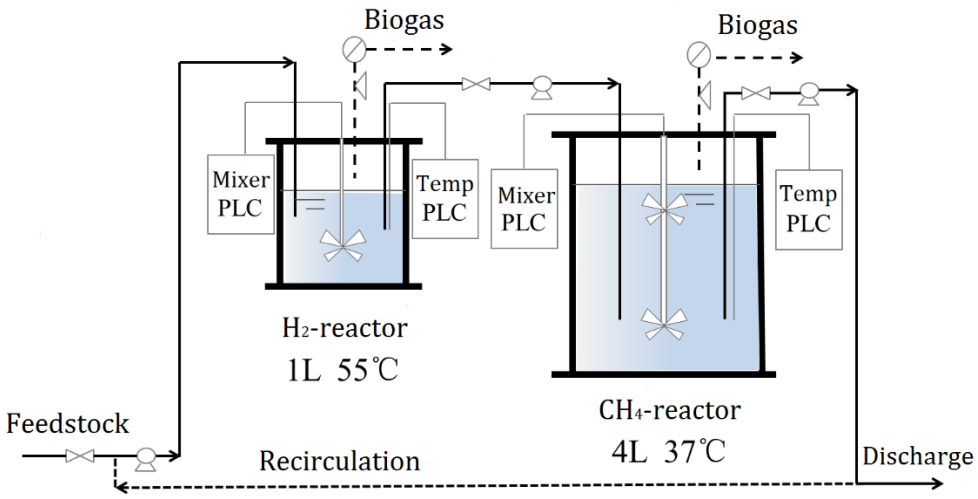


Fig. 1. Schematic of the temperature-separated two-stage anaerobic process.

Table 2
Operating conditions of different periods in the two-stage anaerobic fermentation process.

Operating condition	H_2-reactor		CH_4-reactor		Operating time (d)
	SRT (d)	OLR (g-VS/L/d)	SRT (d)	OLR (g-VS/L/d)	
C-1	1	30.8	4	5.7	1~11
C-2	1	30.3	8	2.9	12~32
C-3	1.2	27.4	12	1.9	33~56
SRR=1:1	1	30.6	4	5.5	1~16
SRR=1:2	1	26.9	4	4.7	17~38
SRR=2:1	1	34.3	4	6.4	39~58

2.3 Analytical methods

The volume of biogas produced in the reactors were measured by gas meter calibration device with U-tube. Compositions of biogas were analysed by a gas chromatograph (BEIFEN 3040, China) equipped with a thermal conductivity detector and a stainless-steel packed column (TDX-01, 2 m). Argon was used as the carrier gas at a flow rate of 35 ml/min. The operation temperatures of the injection port, oven and detector were 100℃, 100℃ and 130℃,

respectively. Ethanol and total volatile fatty acids (TVFAs) including acetate, propionate, butyrate, i-butyrate, valerate and i-valerate in the mixed liquor were analysed by another gas chromatograph (SP6890, China) equipped with a flame ionization detector and a fused-silica capillary column (HP-FFAP, 0.53 mm×10 m×1 μm). Nitrogen was used as the carrier gas with a flow rate of 6 ml/min and split ratio of 10:1. The temperature of injection port and detector were 200℃ and 250 ℃ respectively. Soluble carbohydrate was analysed using anthrone-sulfuric acid method with glucose as standard[7], and soluble proteins was analysed by Lowry method[8]. Chemical oxygen demand (COD) was measured by HACH method (HACH, USA) and pH was measured by glass electrode method (PB-10 Sartorius, Germany). The samples for analysis of soluble carbohydrate and proteins were filtrated by 0.45 μm membrane before detecting. TS, VS, alkalinity and ammonium were determined according to Standard Methods (APHA, 2005).

2.4 Microbial analysis

Discharged sludge samples from H_2-reactor were collected after each period of operation for microbial community analysis. High-throughput sequencing was carried out on Illumina HiSeq platform (Novogene Co. Ltd., Beijing, China). DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., USA) according to the manufacturer's instructions. The primer set 515F (5'-GTGCCAGCAGCCGCGGTAA-3') and 806R (5'-GGACTACCAGGGTATCTAAT-3') was used in the amplification of the V4 region of the bacterial 16S rRNA gene.

3. RESULTS AND DISCUSSION

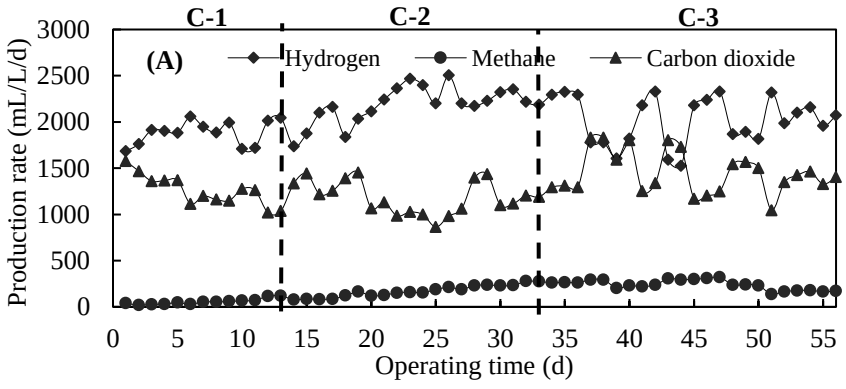
3.1 Biogas production and energy yields

3.1.1 Hydrogen and methane production in H_2-reactor

The production rate of H_2, CO_2 and CH_4 in H_2-reactor with different M-SRT and SRR are shown in Fig. 2. The biogas composition and H_2 yields are shown in Table 3. The biogas in H_2-reactor was mainly composed of H_2 and CO_2 and H_2 yields were close to the result of earlier research (76.8 mL-H_2/g-VS_{added})[6].

Both average production rate of H_2 and H_2 yield increased with higher M-SRT of 8 d. A small amount of methane was also observed in biogas of H_2-reactor, especially with M-SRT of 8 d and 12 d. The production of CH_4 may be contributed to methanogens carried by return sludge from CH_4-reactor. With synergistic interaction between fermenting bacteria and methanogenic archaea[9], interspecies hydrogen exchange can happen in the co-culture system, which means fermentation of glucose to acetate and H_2 in syntrophic association with H_2-consuming microorganisms that keep low partial pressure of H_2[10]. Meanwhile, high partial pressure of hydrogen may inhibit H_2 production[11]. Hence when the hydrogen produced by acetogenic bacteria was consumed by methanogens, especially hydrogenotrophic methanogens, the production of VFAs could be proceeded smoothly. Promotion of CH_4 production with higher M-SRT indicated better activity of methanogens from return sludge. Therefore, H_2 production in H_2-reactor could be promoted by properly prolonging M-SRT. However, H-SRT was relatively extended caused by insufficient return sludge when M-SRT was 12 d. Though with higher activity, the quantity of methanogens from return sludge decreased and thus the CH_4 production of H_2-reactor in period C-3 kept almost the same level with the end of period C-2. Variance of production rate of H_2 and CO_2 increased with higher M-SRT, indicating worse stability of H_2 and CO_2 production. Meanwhile, H_2 yield decreased slightly when M-SRT was 12 d. Possibly the methanogens affected the activity of hydrogen producing acetogenic bacteria and reduced stability of acidogenic stage in the long run. Nevertheless, the H_2 yield was not influenced severely with M-SRT of 12 d.

The production rate of H_2, H_2 content in biogas and H_2 yield both decreased with higher SRR. More seriously, after running for several days when SRR was 2:1, the production rate of H_2 dropped sharply while the production rate of CH_4 increased on the contrary. With the addition of sodium 2-bromoethanesulphonate (BESA, a kind of methanogenic inhibitor) into H_2-reactor, the production rate of H_2 became even higher than that when SRR was 1:2 and the production rate of CH_4 dropped back to original level. The recovery seemed to imply that the collapse of H_2-reactor was due to methanogens from return sludge, especially with higher SRR. The production rate of H_2 and CO_2, mainly substrates for synthesis of CH_4, both decreased corresponding to higher CH_4 production when the collapse happened. Therefore, it might indicate the stage in H_2-reactor transferred from acidogenic stage to methanogenic stage when SRR was 2:1.



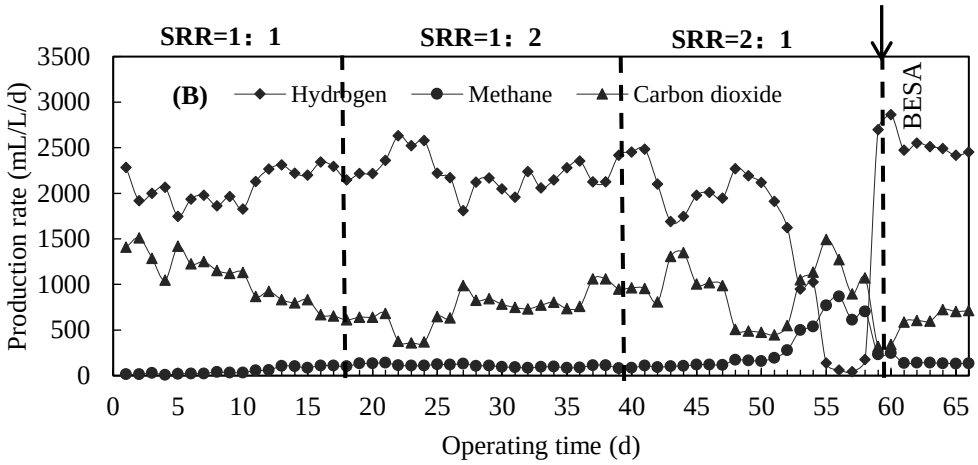


Fig. 2. Production rate of H_2, CO_2 and CH_4 in H_2-reactor with different M-SRT (A) and SRR (B).

Table 3
Biogas composition and hydrogen yields in H_2-reactor under different operating conditions.

Operating condition	Biogas composition (%)			H_2 yield ($mL\text{-}H_2/g\text{-}VS_{added}$)
	H_2	CO_2	CH_4	
C-1	51.0-64.3	34.8-47.8	0.6-2.3	85.5±5.7
C-2	54.9-67.7	26.6-42.4	2.4-7.5	99.8±9.3
C-3	43.0-66.2	29.9-48.7	3.9-8.3	93.1±11.8
SRR=1:1	54.9-75.1	21.4-44.6	0.3-3.6	95.0±8.6
SRR=1:2	61.9-84.4	12.0-33.7	2.8-4.4	102.0±8.8
SRR=2:1	2.7-77.0	17.2-62.2	2.4-39.4	79.5±33.5

3.1.2 Methane production in CH_4-reactor

The sludge from H_2-reactor was discharged into CH_4-reactor. Production rate of CH_4 and CO_2 in CH_4-reactor with different SRR are shown in Fig. 3. The biogas composition and CH_4 yields are shown in Table 4.

The M-SRT were determined by changing the feedstock volume of CH_4-reactor and thus production rate of CH_4 decreased with increasing M-SRT. CH_4 content in biogas and CH_4 yields both increased with higher M-SRT. The highest CH_4 yield, 218.0 $mL\text{-}CH_4/g\text{-}VS_{added}$, was observed when M-SRT was 12 d. Possibly higher M-SRT benefitted the growth of methanogens with low growth rates, such as acetotrophic methanogens[12]. Hence higher activity of methanogens promoted CH_4 production. The change of sludge in H_2-reactor with higher M-SRT might also improve the dissolution and hydrolysis of organic matters, which further boosted CH_4 production in CH_4-reactor.

The stage transfer in H_2-reactor promoted activity of methanogens in H_2-reactor. Then the feedstock of CH_4-reactor, which was from H_2-reactor, promoted CH_4 production in CH_4-reactor and further inhibited H_2 production in H_2-reactor. For the mixed substrates added to H_2-reactor in this study, the volume decreased with higher SRR while the total VS was constant, as mentioned in ***Experimental conditions***. Therefore, VS concentration of the mixed substrate increased with higher SRR, leading to higher VS concentration of feedstock in CH_4-reactor. Production rate of CH_4 in CH_4-reactor remained stable with different SRR and only increased when H_2 production was inhibited in H_2-reactor. Possibly the inhibition of acidogenesis process caused lower degradation rate of organic matters in sludge of H_2-reactor. It provided more biodegradable substrates in feedstock of CH_4-reactor and led to the temporary improvement. The lower CH_4 yields with higher SRR indicated that inhibition of H_2 production in H_2-reactor was unfavourable for CH_4 production in CH_4-reactor. Namely, the hydrogen producing process played an important role in subsequent CH_4 producing stage.

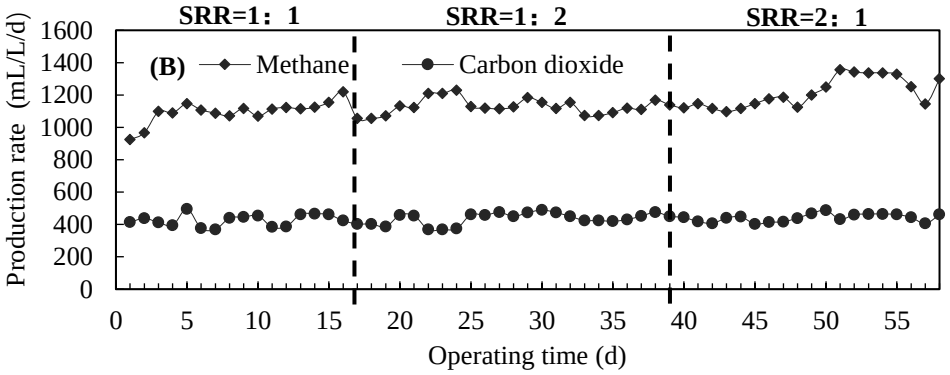


Fig. 3. Production rate of CH_4 and CO_2 in CH_4-reactor with different SRR.

Table 4
Biogas composition and methane yields in CH_4-reactor under different operating conditions.

Operating condition	Biogas composition (%)		Methane yield ($mL\text{-}CH_4/g\text{-}VS_{added}$)
	CH_4	CO_2	
C-1	66.4-71.4	28.6-33.6	187.4±12.5
C-2	67.7-72.0	28.0-32.4	204.4±7.6
C-3	68.6-73.2	26.9-31.4	218.0±13.2
SRR=1:1	68.8-74.6	25.4-31.2	200.2±14.1
SRR=1:2	70.1-76.7	23.3-29.9	240.3±12.8
SRR=2:1	71.4-75.8	24.2-28.6	188.4±18.5

3.1.3 Energy yields

Energy yields of H_2-reactor, CH_4-reactor and total energy yields of the two-stage system under different operating conditions are shown in Table 5. The combustion heat of H_2 and CH_4 are 285.84 kJ/mol and 890.35 kJ/mol respectively[13]. Results show that the main energy yields of the two-stage system came from CH_4. Energy yields of the two reactors were consistent with their respective tending of H_2 and CH_4 production. The total energy yields of the two-stage system increased with higher M-SRT but decreased with higher SRR, reaching maximum value of 8.98 kJ/g-VS_{added} when M-SRT was 12 d and 9.66 kJ/g-VS_{added} when SRR was 1:2 respectively. H_2 production was inhibited slightly while CH_4 production in CH_4-reactor was enhanced with higher M-SRT and thus the total energy yields increased. To some extent, with appropriate concentration of methanogens in H_2-reactor, the energy yields of the two-stage system would be promoted. The energy yield of CH_4 in H_2-reactor increased with higher SRR due to stage transfer. However, with worse production of H_2 in H_2-reactor and CH_4 in CH_4-reactor, the total energy yields decreased with higher SRR. Therefore, excessive methanogens may affect the stability of H_2-reactor and lead to stage transfer, which would inhibit the operation of two-stage system at last.

Table 5
Energy yields of H_2-reactor, CH_4-reactor and total energy yields of the two-stage system under different operating conditions.

Operating condition	H_2-reactor			CH_4-reactor		Total energy yield ($kJ/g\text{-}VS_{added}$)
	Energy yield of H_2 （$kJ/g\text{-}VS_{added}$）	Energy yield of CH_4 （$kJ/g\text{-}VS_{added}$）	Proportion of total energy yield (%)	Energy yield （$kJ/g\text{-}VS_{added}$）	Proportion of total energy yield (%)	
C-1	0.91±0.06	0.07±0.03	12.9	6.56±0.44	87.1	7.53±0.48
C-2	1.06±0.10	0.24±0.09	15.4	7.15±0.27	84.6	8.46±0.29
C-3	0.99±0.13	0.37±0.08	15.1	7.63±0.46	84.9	8.98±0.46
SRR=1:1	1.01±0.09	0.07±0.05	13.3	7.01±0.49	86.7	8.09±0.55
SRR=1:2	1.08±0.09	0.17±0.02	12.9	8.41±0.45	87.1	9.66±0.51
SRR=2:1	0.90±0.35	0.33±0.37	15.7	6.59±0.65	84.3	7.82±0.68

3.2 VS removal efficiency

The VS concentration of discharged sludge from H_2-reactor and CH_4-reactor, VS removal efficiency of the two reactors and total VS removal efficiency of the two-stage system under different operating conditions are shown in Fig.4 and Table 6.

VS removal efficiency of H_2-reactor with M-SRT of 8 d was lower than that with M-SRT of 4 d, while the production of H_2 and CH_4 was promoted. Possibly it was caused by more degradation of carbohydrates and less degradation of proteins and lipids. H_2 yields from carbohydrates were much higher than that from proteins and lipids[14]. The promotion of methanogens in H_2-reactor may affect metabolic pathway of different organics. However, the highest VS removal efficiency in H_2-reactor was 53.3% with M-SRT of 12 d. This result could be caused by the insufficient return sludge from CH_4-reactor mentioned in ***Experimental conditions***. The decrease of feedstock volume led to longer H-SRT, and thus more organic matters could be consumed in H_2-reactor. The VS removal efficiency of CH_4-reactor (21.0%~27.0%) was at the same level of previous research (23.7%-25.1%)[6]. The trend of VS removal efficiency in CH_4-reactor with different M-SRT was contrary to that in H_2-reactor. It might indicate the organics which had not been consumed in H_2-reactor would be used in CH_4-reactor, especially those hardly degraded substrates, such as proteins and lipids. However, most of the biodegradable organic matters in feedstock had been degraded in H_2-reactor and thus limited the utilization of organics in CH_4-reactor. Nevertheless, the VS removal efficiency of the two-stage system increased with increasing M-SRT and reached highest value of 63.8% when M-SRT was 12 d. This result was 17.7% higher than the VS removal efficiency under relatively equal OLR condition (29.3 g-VS/L/d)[6]. It seemed that higher M-SRT promoted the degradation of organics in the two-stage system.

As mentioned in ***Experimental conditions***., VS concentration of the mixed substrate increased with higher SRR, leading to higher VS concentration of feedstock in both reactors. The total VS removal efficiency of two-stage system decreased with higher SRR and the highest total VS removal efficiency of 63.3% was obtained when SRR was 1:2. This trend was consistent with CH_4 production of CH_4-reactor, as well as energy yield of CH_4-reactor and the two-stage system. It might suggest that the temperature-separated two-stage anaerobic fermentation system performed better than temperature-separated methanogenic stage system.

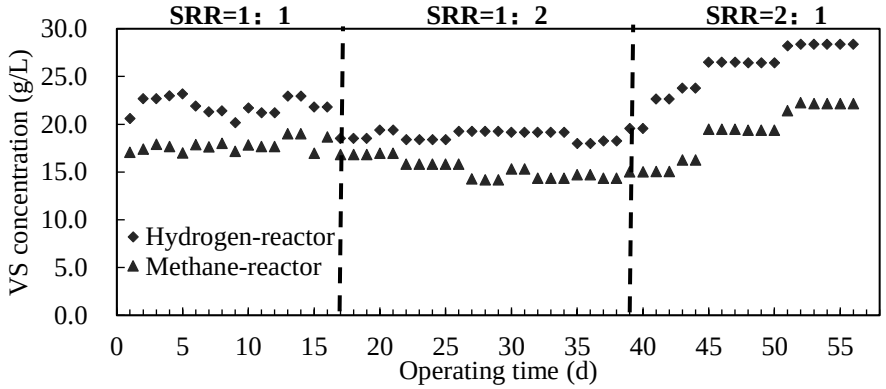


Fig. 4. VS concentration of discharged sludge from H_2-reactor and CH_4-reactor with different SRR.

Table 6

VS removal efficiency and concentration of discharged sludge from H_2-reactor and CH_4-reactor, total VS removal efficiency of the two-stage system under different operating conditions.

Operating condition	VS concentration of discharged sludge (g/L)		VS removal efficiency (%)		
	H_2-reactor	CH_4-reactor	H_2-reactor	CH_4-reactor	Two-stage system
C-1	22.7±0.8	18.0±0.5	50.7±1.4	21.0±2.7	61.1±1.2
C-2	23.1±0.7	16.8±0.7	48.4±2.0	27.0±3.5	62.1±1.9
C-3	22.4±0.7	16.7±0.5	53.3±2.9	25.2±2.2	63.8±1.5
SRR=1:1	Shown in Fig. 4		52.1±1.7	18.9±4.3	61.2±1.4
SRR=1:2			52.2±3.6	22.7±7.7	63.3±1.5
SRR=2:1			51.4±1.0	18.4±2.0	60.6±1.2

3.3 pH and alkalinity

The pH, average alkali dosage and alkalinity of sludge in H_2-reactor and CH_4-reactor under different operating

conditions are shown in Table 7. In two-stage anaerobic fermentation process, return sludge from methanogenic stage can complement alkalinity for acidogenic stage. However, with SRR of 1:1, alkalinity of return sludge cannot meet the demand of acidogenic stage to maintain appropriate pH. In this research, K_2HPO_4 was added into H_2-reactor to maintain pH value of 5.14-5.21, which is in effective range of 5.0-6.5 for hydrogen producing acetogenic bacteria[15]. In CH_4-reactor, pH maintained a suitable range of 7.03-7.06 for methanogens without external alkalinity[16]. All the alkalinity in two reactors were in favourable range of 1000-5000 mg-$CaCO_3$/L[17].

The volume of return sludge decreased with M-SRT of 12 d as mentioned above and the reduction of supplementary alkalinity caused more alkali dosage into H_2-reactor. More acidic substrates such as VFAs produced together with H_2 and more ammonium decomposed from proteins may explained the lower pH and higher alkalinity in H_2-reactor with M-SRT of 8 d compared to that of 4 d. Alkalinity in CH_4-reactor increased with higher M-SRT. Possibly it was caused by the promotion of CH_4 production, which provided better degradation of acidic substrates and more ammonium production.

The average alkali dosage reduced nearly by half with increased alkalinity in H_2-reactor when SRR increased from 1:2 to 1:1. The highest pH value and alkalinity in H_2-reactor was obtained when SRR was 2:1 even with no external alkalinity. Obviously, the return sludge made a great contribution to supplementary alkalinity in acidogenic stage. However, the pH value continued to accelerate over the operating time, up to 5.68 when H_2 production was inhibited apparently. It seems that with higher SRR, return sludge carried not only excessive alkalinity but also more methanogens into H_2-reactor. They promoted CH_4 production and thus caused higher pH value and alkalinity in H_2-reactor, which in turn inhibited H_2 production. Considering the economic cost of external alkalinity as well as running effect, SRR of 1:1 was considered a favorable ratio in this study.

Table 7
pH, average alkali dosage and alkalinity in H_2-reactor and CH_4-reactor under different operating conditions.

Operating condition	H_2-reactor			CH_4-reactor	
	pH	Average alkali dosage (g/L/d)	Alkalinity (mg-$CaCO_3$/L)	pH	Alkalinity (mg-$CaCO_3$/L)
C-1	5.21±0.06	0.791	1951.6±222.5	7.03±0.02	3904.1±124.3
C-2	5.18±0.08	0.922	2103.2±300.9	7.03±0.03	4307.6±225.0
C-3	5.14±0.07	1.640	2376.3±207.4	7.06±0.05	4781.5±255.2
SRR=1:1	5.17±0.04	1.331	2183.7±290.8	7.05±0.03	3771.5±254.6
SRR=1:2	5.11±0.04	2.206	1720.1±68.1	7.01±0.02	3830.2±169.0
SRR=2:1	5.45±0.15	0.000	3054.5±416.3	7.14±0.08	4401.4±315.5

3.4 Soluble organic matters

3.4.1 Soluble carbohydrate, soluble proteins and ammonium

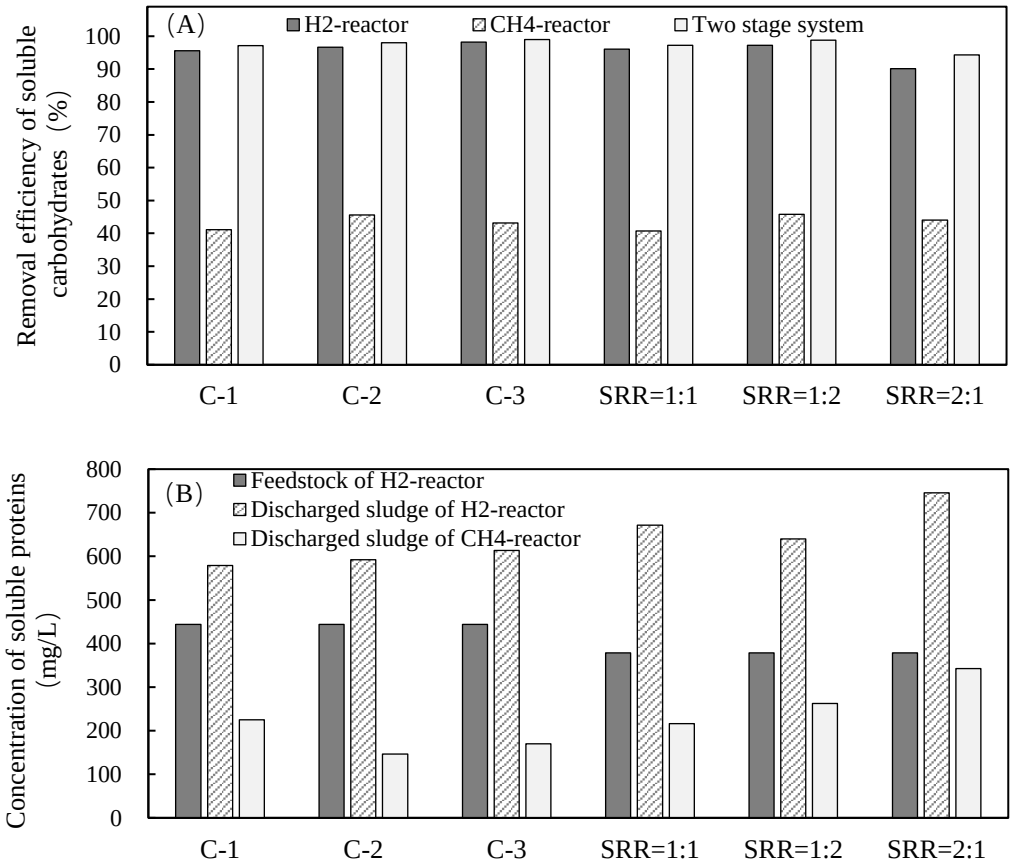
Carbohydrates and proteins are the main biodegradable organic matters in substrates for anaerobic digestion. Removal efficiency of soluble carbohydrates, concentration of soluble proteins and ammonia nitrogen under different operating conditions were shown in Fig. 5.

Most soluble carbohydrates were removed in H_2-reactor with removal efficiency of 95.6%-98.2%. The methane in CH_4-reactor mainly came from the degradation of VFAs as there is few soluble carbohydrates flowed into CH_4-reactor. The slightly increase of removal efficiency of soluble carbohydrates in H_2-reactor with higher M-SRT indicated that with lower stability of H_2 production, the acidogenesis process in H_2-reactor was still promoted. Therefore, removal efficiency of soluble carbohydrates in the two-stage system was promoted with higher M-HRT. Of all the operating conditions, the minimum removal efficiency of soluble carbohydrates in H_2-reactor was observed with SRR of 2:1. Considering the stage transfer in H_2-reactor, it seemed that the utilization of soluble carbohydrates mainly occurred in acidogenic stage in the two-stage system.

Generally, the hydrolysis of macromolecule organics in substrates were considered the rate-limiting step in anaerobic fermentation process. The protein can only be fermented by hydrogen-producing bacteria when it is hydrolysed into amino acids and the utilization depends on its hydrolysis degree[18]. Removal efficiency of soluble proteins in the two-stage system were 40.4%~61.4%, proved that the carbohydrates were more easily biodegraded substrates for anaerobic microorganisms. The concentration of soluble proteins in discharged sludge was much higher than that in feedstock of H_2-reactor, whereas an opposite trend was observed in CH_4-reactor. It is obvious that dissolution rate of proteins from solid phase was much higher than its degradation rate in acidogenic stage. The increasing concentration of soluble proteins in discharged sludge of H_2-reactor with higher M-SRT suggested worse degradation or higher dissolution of soluble proteins. Then the microorganisms in CH_4-reactor broke down the residual proteins which cannot be consumed in

acidogenic stage[19]. Contrary to soluble carbohydrates, the utilization of soluble proteins might be promoted in methanogenic stage rather than acidogenic stage. There was no significant change in removal efficiency of soluble proteins in CH_4-reactor with M-SRT of 8 d and 12 d, suggested a limited ability to degrade soluble proteins in CH_4-reactor. In the SRR optimization experiments, the highest removal efficiency of soluble proteins in CH_4-reactor was obtained with SRR of 1:1. As mentioned above, high SRR of 2:1 caused stage transfer and thus increased the removal efficiency of soluble proteins in H_2-reactor. Therefore, moderate increase of SRR might promote hydrolysis of proteins in CH_4-reactor. But too high SRR would inhibit it and degradation of proteins was inhibited in the two-stage system eventually.

During anaerobic digestion, some organic nitrogen (mainly protein and urea) is converted to ammonia[20]. Ammonia with a proper concentration provides well buffer capacity of methanogenic medium, while high concentration of ammonia may inhibit the activity of microbial and thus disturb the anaerobic process performance[21]. Synopsis of various studies on ammonia inhibition during anaerobic digestion of organic feedstock showed that the inhibition threshold of total ammonia nitrogen (TAN) ranged from 800 mg/L to 14000 mg/L[22]. Therefore, the concentration of ammonia nitrogen was below the inhibition threshold in this study. The higher concentration of ammonia nitrogen in CH_4-reactor with higher M-SRT may be partly influenced by the accumulation of ammonia nitrogen during the whole operating process. Meanwhile, the degradation rate of proteins in CH_4-reactor increased with higher M-SRT and produced more ammonia nitrogen[23]. The concentration of ammonia nitrogen in the two-stage system accumulated obviously with SRR of 2:1. Meanwhile, the concentration of ammonia nitrogen in H_2-reactor and CH_4-reactor were very close, indicating increased hydrolysis of proteins in H_2-reactor when the acidogenic stage transferred to methanogenic stage.



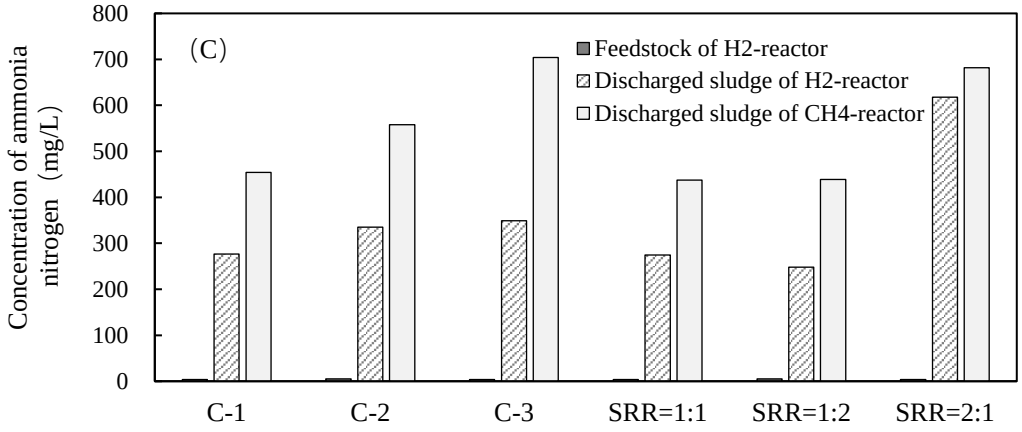


Fig. 5. Removal efficiency of soluble carbohydrates (A), concentration of soluble proteins (B) and ammonia nitrogen (C) under different operating conditions.

3.4.2 VFAs

The concentration of acetate, butyrate and TVFAs in sludge of H_2-reactor and CH_4-reactor under different operating conditions are shown in Fig.6. The main components of TVFAs in sludge of H_2-reactor mainly include acetate and butyrate, implied that acetate or butyrate production pathway were dominant in H_2-reactor instead of ethanol production pathway[24]. The degradation products of butyrate are acetate and hydrogen[25]. Compared with VFAs in H_2-reactor under condition C-1, more butyrate and less acetate as well as improved CH_4 production under condition C-2 implied better metabolism of acetate to CH_4 than butyrate to acetate. The concentration of VFAs in H_2-reactor grew higher under condition C-3. The existence of methanogens in acidogenic stage can enhance the acetate production by consuming H_2 without interfering with the acidogenesis process[12]. Considering worse stability of H_2 production, possibly it was hydrogenotrophic methanogenesis that consumed hydrogen and enhanced acidogenic metabolic pathways. This also caused lower pH and more alkali dosage. The accumulation of both VFAs and alkalinity affected stability of hydrogen producing stage. Most of the VFAs were removed in CH_4-reactor. With higher M-SRT, the VFAs were degraded better and CH_4 production increased correspondingly. Within a certain range, the operation of the two-stage system was enhanced by raising M-SRT. However, higher M-SRT might influence the balance between bacteria and methanogens, and thus reduce the stability of H_2 production. The VS of feedstock in H_2-reactor decreased with lower SRR, led to lower concentration of VFA in H_2-reactor when SRR decreased from 1:1 to 1:2. With SRR of 2:1, the stage transfer in H_2-reactor promoted degradation of VFAs by methanogens and thus the concentration of VFA was lower than that with SRR of 1:1.

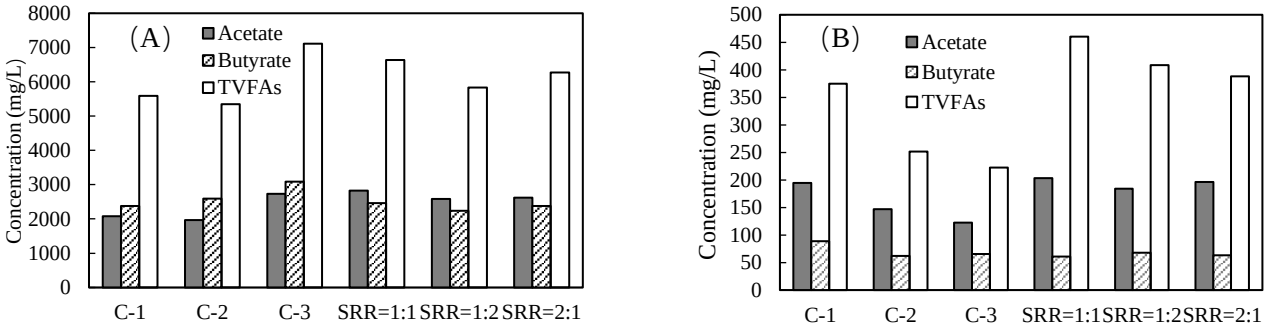


Fig. 6. Concentration of acetate, butyrate and TVFAs of hydrogen (A) and methane (B) producing stage under different operating conditions.

3.5 Microbial analysis

Relative sequence abundances of discharged sludge samples from H_2-reactor at the family level under different operating conditions were shown in Fig. 7. In all the periods, the dominant bacterial families were *Ruminococcaceae* and *Clostridiaceae*, both of which are *Clostridia*. Clostridia usually involve in anaerobic digestion process and perform different essential metabolic pathways, including substrate hydrolysis, fermentation and acetogenesis. They conduct macromolecules hydrolysis[26], such as polysaccharides and oligosaccharides[27], cellulolytic[28] and proteins[29].

Some species of Clostridia also grow in co-culture with methanogens[30] and conduct syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis[31].

Some microorganisms in the family *Ruminococcaceae* are cellulose degrader able to use various sugars, including xylose as a source of carbon[32,33]. *Ruminococcaceae* are also positively correlated with VFAs and metabolism of amino acid and glycan[34]. In particular, *Ruminococcus albus 7* has played a key role in the development of the concept of interspecies hydrogen transfer[35]. The relative abundance of *Ruminococcaceae* were relatively high with higher SRR, indicated the syntrophic association with methanogens. It is suggested that *Ruminococcaceae* are mainly involved in acetate and lactate production/consumption but indirectly contributed to butyrate production via the generation of acetate, lactate, and succinate for interconversion reactions[36].

Studies show that *Clostridiaceae* are related to butyrate[37] and H_2 production[38,39], such as produce H_2 from sugars[40] and lactate[41]. Some species of *Clostridiaceae* are also related to hydrolysis of keratin[42]. The lower relative abundance of *Ruminococcaceae* with higher SRR seemed to suggest the relation between those bacteria and hydrogen production.

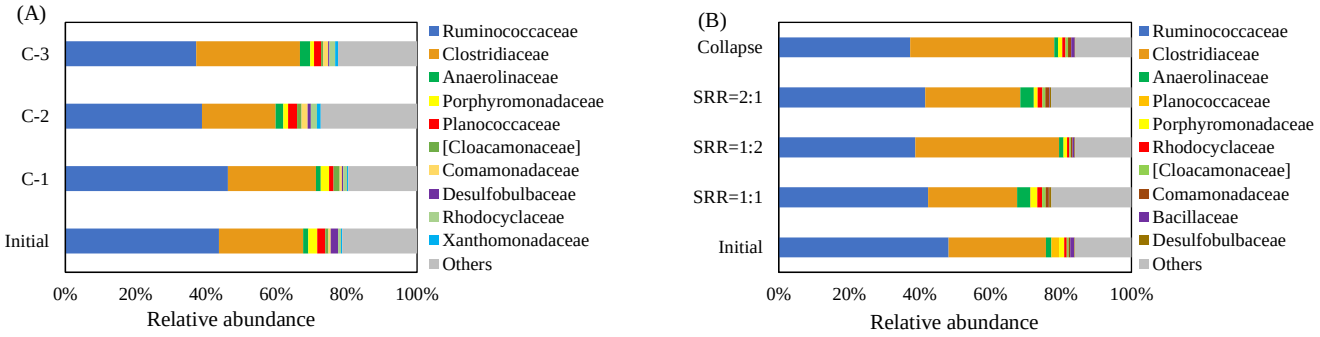


Fig. 7. Relative sequence abundances of discharged sludge samples from H_2-reactor at the family level under different M-SRT (A) and SRR (B).

CONCLUSIONS

In this study, a temperature-separated two-stage anaerobic fermentation process of waste activated sludge and food waste was performed to optimize the M-SRT and SRR. Though the stability of H_2-reactor was affected by higher M-STR, the removal of organics and methane production was promoted for the two-stage system. Proper SRR could complement alkalinity for acidogenic stage while excessive SRR might cause stage transfer and thus inhibit the running of whole system. Therefore, the optimized M-SRT and SRR in this study were 12 d and 1:1 respectively.

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