Journal of Environmental Chemical Engineering 11 (2023) 110224

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

Homoacetogenesis is altering the metabolic pathway of acidogenic microbiome and combating volatile fatty acid accumulation in anaerobic reactors

Zunjing Lv [a,b], Xiaofang Pan [a,b,*], Zhi-Long Ye [a,**], Irini Angelidaki [c], Nan Lv [a,b], Yanlin Li [a,b], Guanjing Cai [a,b], Lifeng Lin [a]

[a] Key Laboratory of Urban Pollutant Conversion, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Technical University of Denmark, Department of Chemical Biochemical Engineering, Lyngby, Denmark

ARTICLE INFO

Editor: Yujie Men

Keywords:
Anaerobic digestion
Homoacetogens
Carbon flow
Ambient temperature
Microbial enrichment

ABSTRACT

Volatile fatty acids (VFAs) (especially propionate and butyrate) accumulation is known to be bottleneck limiting optimal use of anaerobic digestion (AD). Previous studies targeting optimization of the AD process, mainly focused on strategies for enhancing the degradation of propionate and butyrate. In this study, an alternative method to reduce risk of accumulation of higher VFA, was to promote homoacetogens, which are generating small molecules (such as formate, acetate) that can be easily utilized by methanogens by methanogens. Enrichment of homoacetogens would be advantageous for AD process. Two AD reactors operated at two different temperatures (35 ± 1 °C and 24 ± 1 °C) inoculated with heat-pretreated inoculum were used for this study. After 182 days operation, the quantity of homoacetogens, measured by the number of *fhs* gene, representing homoacetogens, reached 1.24 × 10^{14} copies/g wet sludge in ambient temperature (24 °C) reactor, which was much higher than it in mesophilic (35 °C) reactor (1.89 ×10^{12} copies/g wet sludge). The higher copies at ambient temperature compared to mesophilic, was because homoacetogens were surviving at this temperature. At ambient temperature, the acidification rate increased from 30.57 % to 34.25 %, with the composition of acetate and formate of total VFAs raised from 34.86 % to 55.70 %. It meant that enrichment of homoacetogens was resulting in re-routing carbon to methanogenic precursors, which had a significant positive effect on reducing the risk of VFAs accumulation. The genus *Acetobacterium* was the main homoacetogen enriched in 24 °C reactors. The abundance of *Acetobacterium* had a positive correlation with acetate concentration while it had a negative correlation with temperature. This study provides a new approach for mitigation the risk of VFAs accumulation.

1. Introduction

Energy shortage and environmental pollution are the two of the main challenges the world is facing [1]. Recently, 'carbon neutrality' has been proposed to combat climate change, by decreasing carbon emissions [2]. Anaerobic digestion (AD) has been proposed as a promising technology for wastes treatment due to its advantages of producing renewable bio-energy from multiple organic substances [3]. Methane has high calorific value (55.7 KJ/g) and less CO_2 emissions when it is burned [4]. The AD is a complex process consisting of hydrolysis, acidogenesis, acetogenesis and methanogenesis [5,6]. The imbalance between the acidogenesis and methanogenesis in AD systems, is in the volatile fatty acids (VFAs) accumulation, especially propionate and butyrate. Propionate and butyrate are converted through acidogenic reactions, to acetic acid and H_2. These oxidation reactions are thermodynamically unfavorable at standard conditions and can only take place at significantly low product concentrations of products, in order to turn the conversion reaction to exergonic. The accumulation of propionate and butyrate could lead to the pH decline [7] and further inhibited the biogas production and CH_4 yield [8]. In order to solve the problem of imbalance between the acidogenesis and methanogenesis, previous research [9] proposed separation of AD process into two-stages to provide

* Corresponding author at: Key Laboratory of Urban Pollutant Conversion, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China.
** Corresponding author.
E-mail addresses: xfpan@iue.ac.cn (X. Pan), zly@iue.ac.cn (Z.-L. Ye).

https://doi.org/10.1016/j.jece.2023.110224
Received 18 March 2023; Received in revised form 15 May 2023; Accepted 27 May 2023
Available online 29 May 2023

appropriate environment for acidogens and methanogens respectively. However, the VFAs generated from acidogenic phase containing a large proportion of propionate and butyrate which hardly to be utilized by subsequent methanogenic phase.

Reducing accumulation of propionate and butyrate, to provide methanogenic substrates, has been intensively studied [10,11], and several methods have been proposed, such adding granular activated carbon (GAC) or biochar to prompt the direct interspecies electron transfer (DIET) [12,13], bioaugmentation of syntrophic fatty acids oxidizing bacteria and methanogens [14], operating parameters regulation (pH, ORP, HRT etc.) [15,16]. However, these initiatives could not completely and stably avoid VFA accumulation, when the process was subjected to external unfavorable environmental conditions or substrate load shocks. Therefore, development of effective and radical solution to acids accumulation problems is still a topic of great importance. Strengthening production of acetate and formate by enrichment with acetogenic microorganisms in AD system could result in a stable and efficient way to avoid VFA accumulation.

Homoacetogens, a diverse group of strictly anaerobic microorganism which could utilize a wide variety of substrates to generate acetate, growing autotrophically by consuming CO_2/H_2 to produce acetate via Wood-Ljungdahl pathway (WLP) [17] and growing heterotrophically by utilizing organic matter such as single organic sugar (glucose), acid (formate, lactate) and alcohols [18]. Enriching homoacetogens in the reactor can reduce the generation of VFAs with more than 3 carbon atoms, such as propionate and butyrate from the origin. Although some previous studies enriched homoacetogens at a mesophilic (35 ± 1 °C) reactor with the supplement of methanogens inhibitor BES [19,20]. A few studies focused on the role of homoacetogens enrichment on shifting the metabolic carbon flows and microbial successions in the AD system.

Therefore, we attempted to enrich homoacetogens in continuous stirred-tank reactors (CSTR) at both mesophilic (35 ± 1 °C) and ambient (24 ± 1 °C) temperature. The objectives of this study were: (1) to evaluate the effect of homoacetogens on the acidification rate and carbon flow; (2) to reveal the effect of enrichment by homoacetogenic bacterial on the microbial community composition and the metabolic network, at different operation phases. To achieve these goals, two CSTRs were inoculated with acidogenic inoculum (heated sludge) and fed with different substrates. This study could provide an alternative option for mitigating the risk of VFAs accumulation at the source by enriching functional groups and further altering carbon flow in acidogenic phase.

2. Materials and methods

2.1. Inoculum, reagents and bioreactor

The inoculation anaerobic sludge was obtained from a sewage treatment plant in Jimei, Xiamen City, China. The parameters of seed sludge were measured: pH 6.8, mixed liquid suspended solid (MLSS) 42.4 g/L, mixed liquid volatile suspended solids (MLVSS) 13.9 g/L. Before inoculation, the sludge was initially filtered through a 50-mesh strainer to remove debris. Then intermittent culture and acclimation were conducted in a 5 L anaerobic fermentation bottle by dosing appropriate glucose amount. After acclimation, the sludge was heated at 100 °C for 2 h to kill the methanogens for avoiding their consumption of VFAs (principally acetate and formate) [21], then was put in the 5 L bottle again to activate 48 h with glucose at 37 °C.

Different synthetic substrates were prepared for homoacetogens cultivation with addition of various carbon source. The carbon source was glucose at the start-up phase, while it was changed to a mixture of glucose and formate at the transition phase, and formate at the enrichment phase (Table 1). NH_4Cl and mixture of K_2HPO_4 and KH_2PO_4 (1:1) were added as the nitrogen source and phosphorus source to keep the mass ratio of COD: N: P at 200: 5: 1. Trace metals and minerals solution were prepared and supplemented to enhance the activity of inoculated sludge, and the detailed information about these solutions was referred to Lv et al.' s research [1].

The CSTR was constructed by polymethyl methacrylate with working volume of 8 L (Fig. 1). There was a water bath layer around the outer wall of CSTR, equipped with a temperature control system (THS-10, Tianheng, China) to ensure the temperature of 35 ± 1 °C or 24 ± 1 °C, respectively. The influent was fed to reactor through a peristaltic pump (BT-100 S, Lead Fluid, China) at a fixed flowrate (8.5 rpm/min).

2.2. Start-up and operation phases of CSTR

At the start-up phase, 2.5 L seed sludge was quickly added into reactor and then the equipment was sealed strictly to ensure the react zone in a rigorous anaerobic state. Two CSTRs were started up and operated at mesophilic (35 ± 1 °C) and ambient conditions (24 ± 1 °C), respectively. The organic loading rate (OLR) was stepwise increased from 3.0 to 12 kg COD/(m^3■d) by increasing COD concentration of influent at a stable HRT of 8 h. The pH was adjusted to suitable acidic condition with the supplementation of $NaHCO_3$ solution. As shown in Table 1, the operational process of CSTR system included three main phases: start-up phase (P1-P3), transition phase (P4-P6) and enrichment phase (P7-P9). At P10, glucose was added as substrate for evaluating the performance of acidification after homoacetogens enrichment.

2.3. Analytical methods

The parameters of MLSS, MLVSS, alkalinity and COD concentration were measured by referring to the Standard Methods (APHA, 2012). pH and oxidation-reduction potential (ORP) were detected by multichannel electrochemical tester (HQ40D, HACH, USA). Before analyzing the volatile fatty acids (VFAs), liquid samples collected from the CSTR were firstly centrifuged at 6000 rpm for 5 min, then filtered through a 0.22 μm filter membrane and acidified to pH< 2 by dosing 5 mol/L HCl. The concentrations and compositions of VFAs, including lactic acid, formic acid, acetic acid, propionic acid, iso-butyric acid, n-butyric acid, isovaleric acid and n-valeric acid, were detected by using ion chromatography (ICS-3000, Dionex, USA) equipped with an anion exchange column (Dionex IonPac™ AS11). Biogas collected from the CSTR was measured by a wet gas flowmeter (LMF-1, China) and the compositions of H_2, CH_4 and CO_2 were analyzed by the gas chromatography (GC-9790II, Fuli, China) equipped with packed column (TDX-01) and thermal conductivity detector.

Regarding the acetogenesis efficiency (AE, %), it was calculated as:

$$AE = \frac{COD_{HAc}}{COD_{Influent}} \times 100\%$$

Table 1
Operation parameters of CSTR.

Phase	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Operation time (d)	1–20	21–36	37–52	53–68	69–88	89–107	108–126	127–143	144–162	163–182
Carbon source	Glucose			Glucose+Sodium formate			Sodium formate			Glucose
Concentration (g/L)	1.0	2.0	4.0	3.76/1.0	3.53/2.0	3.23/3.25	3.25	6.5	13.0	4.0
OLR (kg COD/(m^3■d)	3.0	6.0	12.0	12.0	12.0	12.0	2.31	4.62	9.24	12
HRT (h)	8	8	8	8	8	8	8	8	8	8

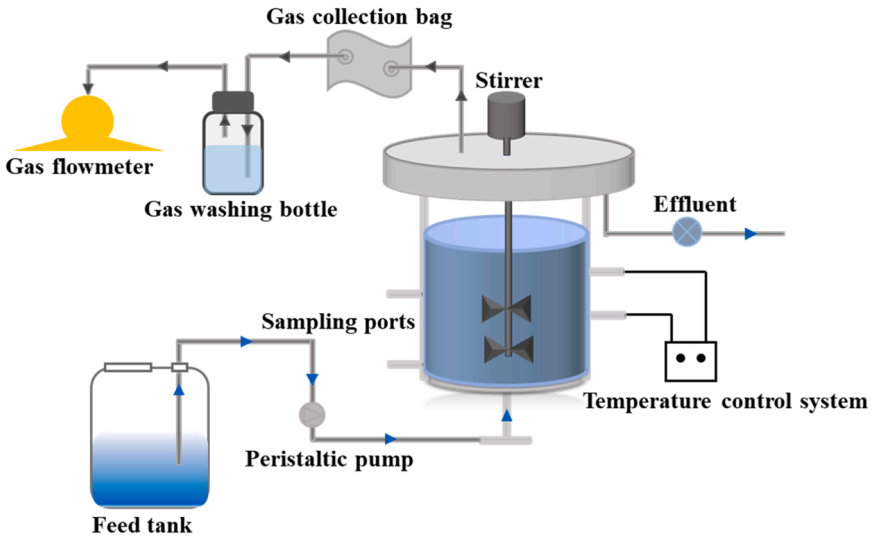

Fig. 1. Schematic diagram of the CSTR system.

where COD_{HAc} is the concentration of effluent acetic acid (mg COD/L), $COD_{Influent}$ is the concentration of influent (mg COD/L).

2.4. Microbial analysis

2.4.1. DNA extraction and PCR amplification

Sludge samples were taken out from reactors at designed interval times and then were stored at − 80 °C for microbial analyses. The extraction of DNA from collected sludge samples was carried out by FastDNA™ spin kit (mpbio™, USA) following the operating steps in instruction manual. The concentration and purity of extracted DNA were measured by NanoDrop-2000 spectrophotometer (Thermo Fisher Scientific, USA). Primers FHS-1 (5′-GTWTGGGCWAARGGYGGMGAAGG-3′) and FTHFS-r (5′-GTATTGDGTYTTRGCCATACA-3′) were then used for the PCR amplification of formyltetrahydrofolate synthetase (*fhs*) [22]. All oligonucleotide primers were synthesized by the Accurate Biotechnology Co. (Hunan, China). The volume of each PCR amplification mixture was 50 μL, containing 25 μL of 2 ×Pro Taq Master Mix (dye plus), 1 μL forwards primer, 1 μL reverse primer, 2 μL DNA Template and 21 μL RNase free water. PCR reactions were running on a T 960 C PCR amplifier (Heal Force, China), which has been described by [22].

2.4.2. qPCR analysis

For qPCR of *fhs* gene, the volume of each reaction mixture was 20 μL, containing 10 μL 2 ×SYBR Green Pro Taq HS Premix, 0.4 μL forwards primer, 0.4 μL reverse primer, 7.2 μL RNase free water and 2 μL diluted DNA sample (approximately 10 ng/μL) or plasmids, respectively. The negative control was needed in qPCR analysis, where ultrapure water was used to replace DNA samples. Each reaction mixture was performed in triplicate and all the mixtures were added in the 96-well plates, then centrifuged at 3000 rpm/min for 2 min before analysis. The program of qPCR was referred to the literature [22], which was performed by LightCycler 480 RT-PCR system (Roche Diagnostics GmBH, Germany).

2.4.3. 16 S rRNA illumina sequencing

The microbial communities of sludge samples collected from both CSTRs were analyzed by high-throughput sequencing with the Illumina Miseq platform. A set of universal primers 515FmodF (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806RmodR (5′-GCACTACNVGGGTWTCTAAT-3′) were used for the amplification of V4 region of 16 S rRNA gene in each sludge sample [3]. The data analysis of 16 S rRNA was processed through Majorbio I-Sanger Cloud Platform (www.I-sanger.com). The relative abundance of functional genes of different operational phases were predicted based on OTU abundance applying the PICRUSt2 software.

3. Results and discussion

3.1. Performance of CSTR operation

3.1.1. Operation parameters and acetogenesis efficiency

The details of operation parameters containing the pH, ORP and alkalinity were presented in Fig. 2(a)–(c). For a better directional enrichment, different substrates with different concentrations were utilized during different operational processes. Thus, the CSTR system would have an adaption stage of 10–15 days with these parameters showing fluctuation, and then it tended to be stable gradually after adaption stage. The pH of both reactors was adjusted by $NaHCO_3$ to meet optimal pH value for acidogenesis and homo-acetogenesis. According to Tsapekos et al.' s study [23], the optimum pH range for the growth of homoacetogens was 5.5–7.0. When glucose was used as the sole substrate, the pH was stable at 5.0–6.0. Addition of sodium formate raised pH at P4-P6 due to alkalic reaction of the sodium formate salt. Thus, at P7, the formate was added solely without any addition of $NaHCO_3$, the pH increased to above 9.0. At P8-P9, the formic acid was supplemented to prevent the pH increased further. The ORP value of both reactors had fluctuation when changing the substrate of influent. Overall, the ORP of both reactors were maintained below − 200 mV. Therefore, both reactors were operated at a strictly anaerobic condition.

Alkalinity is considered as an important parameter to maintain stability in the AD system [24]. The alkalinity of both reactors was kept stable at around 600 mg $CaCO_3$/L with 1.5 g/L $NaHCO_3$ at P1, P2 and P10. The alkalinity showed improved tendency with the increased OLR at P3 and increased further to above 2300 mg $CaCO_3$/L with the formate addition during P4-P6. Then decreased at P7 without the addition of $NaHCO_3$. In line with the pH variations, the alkalinity of ambient temperature reactor (R-amb), began to decrease more than the mesophilic one (R-meso). Generally, the alkalinity indicated a better buffer capacity and stability of the reactor.

Acetogenesis efficiency (AE) reflects the acetogenesis ability of anaerobic systems. As shown in Fig. 2, the AE of the R-meso fluctuated and displayed a higher level than that of R-amb, which was due to the higher degradation degree of glucose mesophilic temperature. According to Schmidt et al.' s study [25], organic matter degradation rate in AD process is increasing at higher temperatures. That might be the reason that AE in mesophilic reactor was relatively higher than it in ambient one. In addition, it should be noted that AE values in P7-P9 were decreased to almost zero, and recovered to normal level in P10 phase,

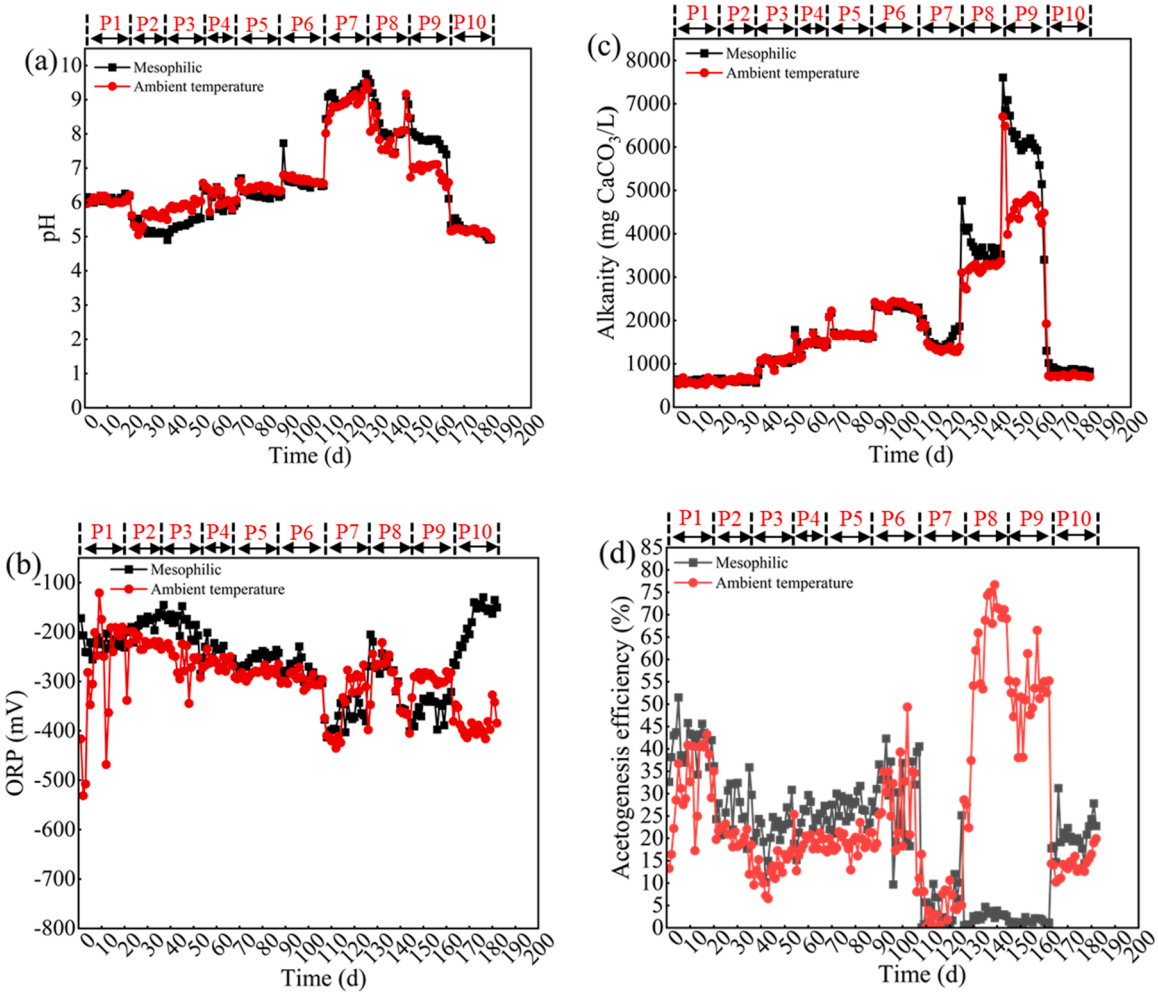

Fig. 2. Variation of daily pH (a), ORP (b), alkalinity (c) and acetogenesis efficiency (d).

indicating that formate had not been used to produce acetate in the mesophilic system. Instead, other formate consuming processes e.g hydrogenotrophic methanogenesis, was the dominant formate consumption pathway (more detailed discussion in Section 3.1.3). On the contrary, R-amb, significant enhancement of AE was observed at P7-P9, which was due to sodium formate in this system was successfully utilized by homoacetogens which were enriched at these conditions.

3.1.2. *VFAs production*

Fig. 3 dipicts the composition of VFAs during the whole experiment duration. It was clear that the production of acetate was enhanced by increasing dosage of sodium formate at P4-P6. Li et al. [18] reported that, except glucose, homoacetogens could use formate as substrate to generate acetate. Significant differences existed between the two reactors at P7-P9. The yield of acetate in both reactors were kept at a relatively low level, around 100 mg/L at P7, due to the high pH value above 9.0 limited the homo-acetogenic process, since the homoacetogens are growing at pH range of 5.5–7.0 [23]. When formic acid was added to adjust the pH level, the yield of acetate increased at the ambient temperature. It reached to around 1000 mg HAc/L at P8 with 6.5 g/L formate and 1500 mg HAc/L at P9 with 13.0 g/L formate, while it still remained at relative low level at the R-meso during P7-P9. These results indicated that acetate increase could mainly be ascribed to homoacetogenesis from formate at ambient temperature [26]. However, the majority of formate in mesophilic reactor was consumed by revived hydrogenotrophic methanogens to generate CH_4 instead of acetate, mainly responsible for the low yield of VFAs. This result is suggesting that homoacetogens outcompeted methanogens, for formate use at low temperature [27].

In order to evaluate the effect of enrichment with homoacetogenic bacteria on acidification, the performance of acidogenesis with the same substrate of glucose, before enrichment at P3 and after enrichment at P10 was compared and discussed in both R-amb and R-meso reactors. Generally, the proportion of acetate in each reactor was significantly increased after enrichment, with value increasing from 39.39 % to 48.33 % in R-meso and from 32.49 % to 45.98 % in R-amb. This result was in line with expectations that homoacetogens enrichment contributed to high acetate generation and low CO_2 emission. Besides acetate, butyrate was another dominant VFAs increased in both reactors, accounting 36.03 % of total VFAs in R-meso and 47.01 % in R-amb. Most importantly, the contribution of propionate to total VFAs showed significant reduction in two reactors after enrichment, namely decreasing from 20.75 % to 2.34 % in R-meso and 8.76–0.33 % in R-amb. Based on thermodynamic calculations it is evident that syntrophic propionate oxidization requires more energy:

$$CH_3CH_2COOH+H_2O \rightarrow 2CH_3COOH+3\ H_2+CO_2\ (\Delta G^0 = +76{\cdot}67\ kJ/mol)\ (1)$$

$$CH_3CH_2CH_2COOH+2\ H_2O \rightarrow 2CH_3COOH+2\ H_2\ (\Delta G^0 = +48{\cdot}30\ kJ/mol)\ (2)$$

[28]. Reducing production of propionate during acidogenesis process had advantage in relieving acids accumulation [29]. Therefore, it can be concluded that the enrichment with homoacetogens in period P4-P9 reduced the risk of higher VFAs accumulation and subsequently positively affected methanogenesis. In addition, another interesting

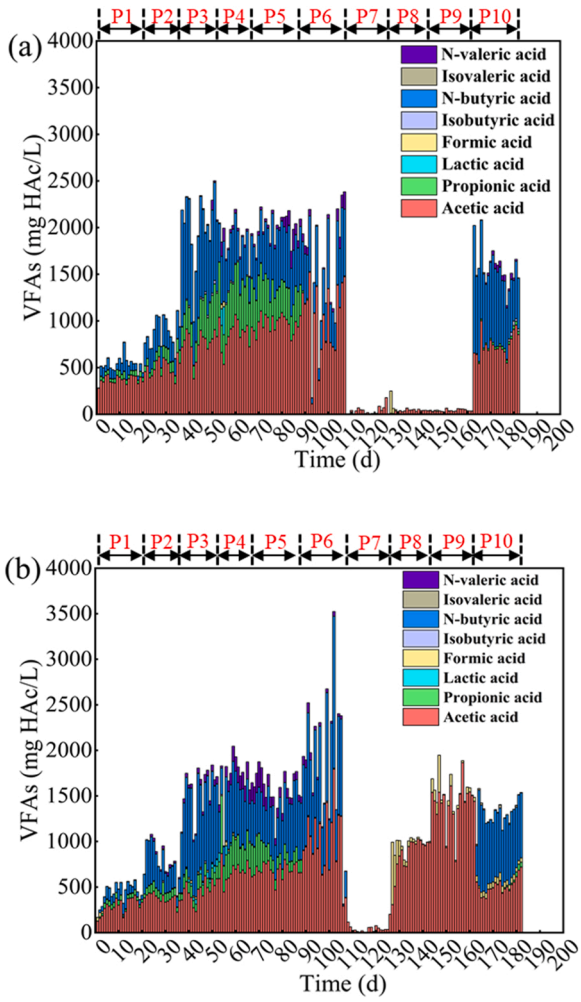

Fig. 3. Profiles of VFAs production in mesophilic (a) and ambient temperature reactor (b).

observation was that around 60–70 mg formate expressed as HAc-equivants/L was detected in R-amb, accounting for 5.99 % of total VFAs. This was not expected, but nevertheless it is beneficial for the subsequent methanogenesis, since formate was an important substrate for methanogens. Formate has been reported to be easier taken up as methanogenic substrate compared to H_2 [30,31]. According to Nolling et al., (2001)'s report, pyruvate formate-lyase (PFL), the functional enzyme responsible for the formate generation, is a strict anaerobic enzyme [32]. Therefore, the formate production is more favorable under low ORP condition. This result is accord to the lower ORP value of P10 at R-amb as demonstrated in Fig. 2b. In addition, the produced formate could accumulate in the R-amb without the consumption of methanogens."

3.1.3. Gas production

As shown in Fig. 4, low gas was produced in R-amb during P1-P9 compared to R-meso. When formate was supplemented at P4-P6, the H_2 cleavage from formate partly consumed by methanogens to generate methane, others utilized by homoacetogens to generate acetate, which raised the proportion of acetate presented in the Table. A.1.

With formate as the sole substrate during P7-P9, the yield of CH_4 was increased remarkably in R-meso, suggesting that most formate was consumed by methanogens, which shows that methanogens were reactivated. This proves that pretreatment of sludge could not permanently inhibit the methanogenesis which is in agreement with previously shown by [33]. In contrast, only low CH_4 production was observed in R-amb. This result indicated that the temperature was a key factor, which influencing the competition between homoacetogens and methanogens for formate consumption.

With the addition of glucose at P10, H_2 and CO_2 were regenerated and accounted for a larger proportion of total gas production in R-meso and R-amb. Notably, the gas production presented downtrend in both reactors. As showed in Table. A.1, the proportion of acetate in R-meso and R-amb raised from 34.82 % and 28.84 % (P3) to 60.51 % and 52.11 % (P10) respectively. Therefore, the homoacetogenic pathway promoted the generation of acetate [34,35].

3.1.4. Carbon flow shifting

In order to elucidate the effect of the enrichment of homoacetogens, the difference in the carbon flows between two reactors after the enrichment was analyzed. The carbon flow analysis was carried out in the stages of P3 (before cultivation) and P10 (after cultivation) in both reactors, respectively. As demonstrated in Fig. 5, the carbon originated from glucose was defined as 100 %. In the P3 of R-meso, approximately 48.12 % of the carbon flowed to VFAs including 21.98 % of butyrate, 5.71 % of propionate and 20.25 % of acetate. After enrichment in P10, the proportion of acetate increased to 22.33 %, while the butyrate and propionate decreased to 10.75 % and 0.76 % respectively. Similarly, in R-amb, the enrichment increased the proportion of acetate and formate to 19.08 % (P10) from 11.94 % (P3), and less carbon was flowing to propionate, which reduced propionate portion from 2.39 % (P3) to 1.24 % (P10), while still 13.93 % of the carbon went to butyrate in period P10 compared to the 16.12 % in P3.

Notably, the carbon flowed to total VFAs and gas were 48.2 % and 3.3 % respectively, more than half carbon conversions were unknown. Partly carbon might be directly utilized by microbials for growth and reproduction. Besides, according to Ahn et al., (2006)'s study, some controlling parameters such as pH and temperature can limit the acidogenic rate of AD system [36]. The optimal pH value for hydrolysis and acidification was probed at natural or 9.0 [37,38]. Short chain VFAs were more easily produced as final products at higher pH condition. Except the VFAs and gas, carbon also could transfer into other organic substances such as ethanol, butanol and acetone, the proportion was highly related to the fermentation style [39]. The rules of carbon conversion during different anaerobic digestion style are deserve to explore in the future.

Generally, the enrichment with homoacetogens, led to more carbon flowing to small molecules including formate and acetate, while lower portion of carbon was flowing to propionate. In agreement with the results demonstrated in Section 3.1.2 showing that the proportion of acetate in R-amb improved to 45.98 % (P10) from 32.49 % (P3), while the proportion of propionate and butyrate reduced to 0.33 % and 47.01 % (P10) from 8.76 % and 53.17 % (P3).

3.2. Microbial community analysis

3.2.1. Diversity and relative abundance of archaea and bacteria at different phases

The diversity indices (Shannon, Simpson) and richness indices (Ace, Chao) were used to analyze the diversity and richness of microbial community [40]. As shown in Table. A.2, the coverage index of all sludge samples was more than 99 %, suggesting that the microbial community could be effectively covered by the high-quality sequence database constructed from each sludge sample. The richness (Ace, Chao) and diversity (Shannon, Simpson) of original sludge (P0) was higher than the ones, during operation of the reactors. Both richness and diversity decreased gradually from P1 to P9, except a slight increased at P10 when glucose was the substrate. It is expected that single, simple carbon sources are reducing the microbial diversity and richness of a microbiome [41].

To reveal the dynamic succession of microorganisms and main functional microbes at different phases in R-meso and R-amb, the

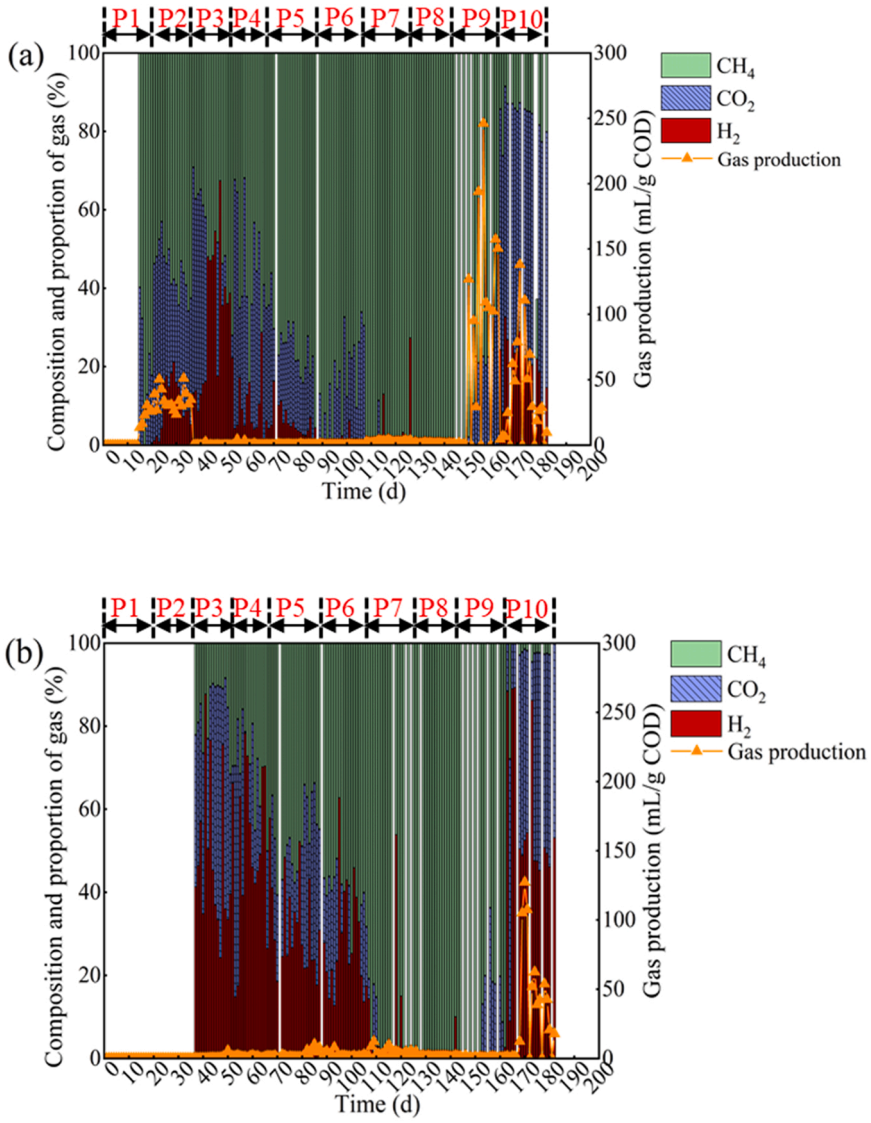

Fig. 4. Evolution of gas production (hydrogen, carbon dioxide, and methane), mesophilic reactor: (a), ambient reactor: (b).

composition and relative abundance of microorganisms were analyzed at phylum and genus level (Fig. 6). The most dominant phylum of original sludge in both reactors was *Firmicutes*, accounting for 79.88 % and 67.91 % in R-meso and R-amb reactors, respectively. This phylum is the abundant phylum in wastewaters, solid waste and sludges from AD system and responsible for butyrate generation [9,42]. This result was consistent with butyrate as dominant VFAs as described in Fig. 3. At P1-P3, *Firmicutes*, *Proteobacteria* and *Bacteroidetes* were the major bacterial phyla in both reactors, these three phyla are commonly hydrolysis and acidification microorganisms in AD systems [43]. *Bacteroidetes* participated in the biodegradation of carbohydrates, proteins and peptides [44]. Their abundance was positively correlated with glucose added, while it decreased with formate. *Firmicutes* and *Proteobacteria* could utilize glucose as substrate, and *Firmicutes* were mainly responsible for the production of butyrate and acetate, while *Proteobacteria* mainly contributed to the generation of acetate [39,45]. Their high relative abundance may result in the large proportion of acetate and butyrate. Moreover, as previous research, the carbonate alkalinity stress was closely related to the abundance of *Proteobacteria* and *Firmicutes*. The relative abundance of *Proteobacteria* showed increased tendency while the *Firmicutes* abundance declined with increasing alkalinity level [46]. Yang et al., (2019) reported that the abundance of *Proteobacteria* was significantly downregulated and the abundance of *Firmicutes* raised with increasing alkalinity [47]. Briefly, there is a competitive relationship between the *Firmicutes* and *Proteobacteria* with increasing level of alkalinity. Thus, the higher alkalinity as presented in Fig. 2(c) may lead to the negative correlation between *Firmicutes* and *Proteobacteria* [47] during P1-P6 at both reactors. Then, with formate addition at P7-P9, *Euryarchaeota*, belonging to archaea, known for involvement in methane generation [48], reactivated in the mesophilic reactor and replaced the dominant of hydrolysis and acidification microorganisms. However, in R-amb, the acidification microorganisms still accounted for a larger proportion. This result provides evidence for the low acetate production and regeneration of methane during P8-P9 in mesophilic reactor.

On genus level, *Clostridium_sensu_stricto_1* and *unclassified_f_Enterobacteriaceae* were the dominant microbes during P1-P3. *Clostridium_sensu_stricto_1*, belonging to phylum *Firmicutes*, is a genus of acidogenic bacteria commonly found in anaerobic systems, which could utilize carbohydrate and proteinaceous substances as carbon source to produce acetate, butyrate, propionate and H_2/CO_2 [6,49]. In both reactors, the relative abundance of *Clostridium_sensu_stricto_1* was increased with the increased dosage of glucose (P1-P3) and its increase in relative abundance corresponded well to the observed accumulation

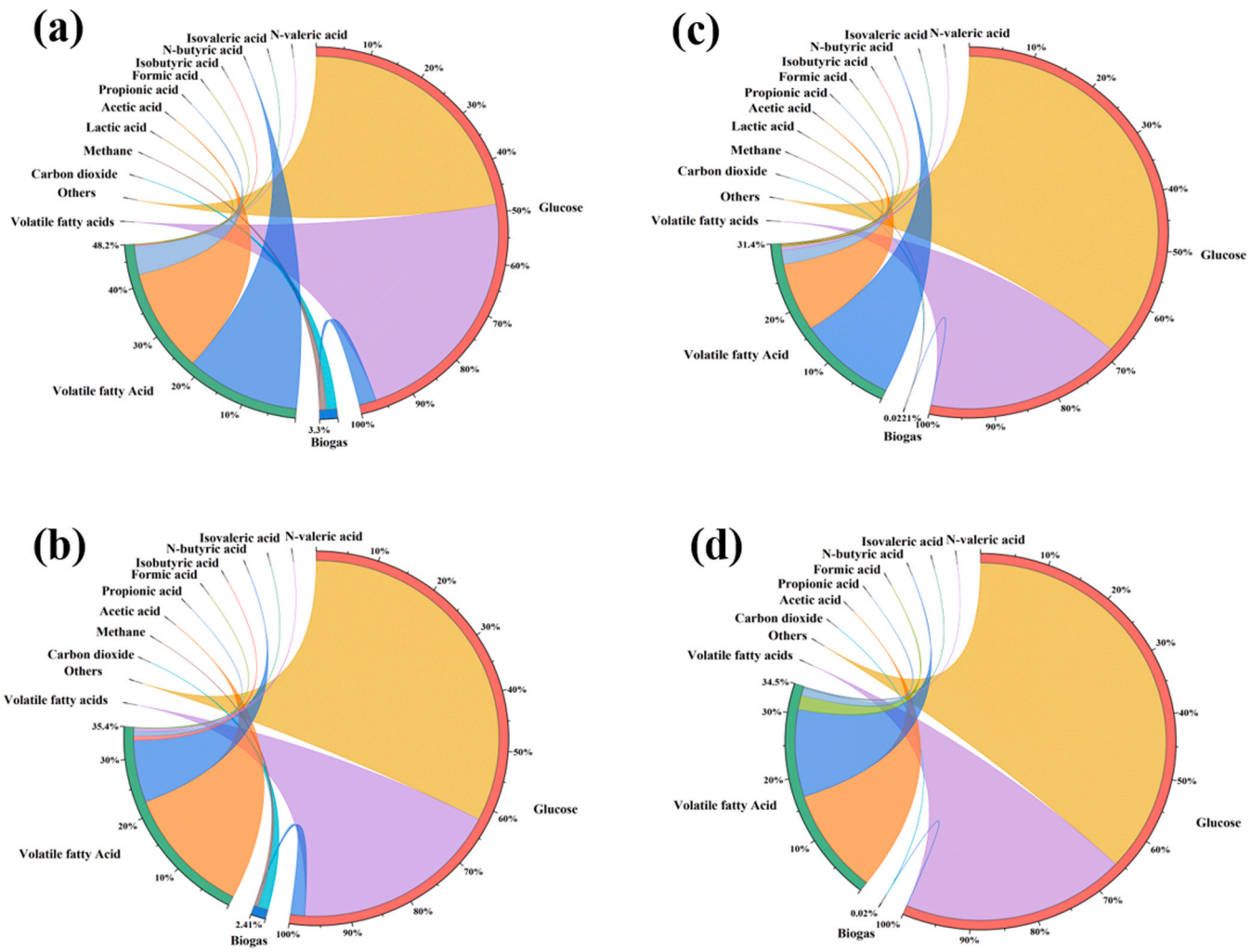

Fig. 5. Variation of carbon flow before (P3) and after (P10) homoacetogens enrichment: mesophilic reactor at P3 (a); mesophilic reactor at P10 (b); ambient reactor at P3 (c); ambient reactor at P10 (d).

of VFAs. Otherwise, *unclassified_f_Enterobacteriaceae*, known for fermenting sugars such as glucose and lactose, also related to the generation of VFAs [50], was the second abundant genus during the periods P1-P3, while it decreased sharply when formate was the carbon source and was thereafter present at a relative lower abundance at P10 in both reactors. This could explain the reduced VFA concentrations. *Desulfovibrio*, is highly related to the production of acetate [51]. Its relative abundance was increased when formate was added in P4-P6. *Acetobacterium*, known as the homoacetogens, could convert H_2/CO_2 to acetate autotrophically according to following equation: $4\,H_2 + 2CO_2 \rightarrow CH_3COOH + 2\,H_2O$ [52], also could generate acetate via fermenting glucose, formate and lactate heterotrophically [53]. Higher relative abundance of *Acetobacterium* was detected in R-amb at formate-adding phases during P8 (37.52 %)-P9 (29.64 %) than it at P7 (0.15 %) and scarcely detected at former 6 phases (Fig. 6d). Therefore, the enrichment of *Acetobacterium* and low abundance (1.05–3.78 %) of hydrogenotrophic methanogens *Methanobacterium* [1,54] during P7-P9 contributed to the increased acetate production in R-amb. As a contrast, the relative abundance of *Methanobacterium* reached the highest level at P8-P9 with proportion of 76.08–86.80 % in R-meso.

In summary, the diversity and relative abundance of microbes showed a distinct variation at the different phases. The homoacetogens *Acetobacterium* was enriched in R-amb, suggesting that the temperature plays a key role on the enrichment [27]. The enrichment of the homoacetogenic bacterium *Acetobacterium* in AD reactors not only can reduce the CO_2 production, but also have the ability to convert glucose to acetate.

3.2.2. Correlation between microbial abundance and bioreactor environment

Considering the environmental conditions are highly related to the microbial community composition, redundancy analysis (RDA) was employed to analyze correlations between microbial community with temperature, and concentrations of formate and acetate in the reactors. As shown in Fig. 7, RDA1 and RDA2 accounted for 28.90 % and 11.52 % of the general variation of microbial community composition, indicating that RDA1 played a leading role in microbial distribution. It also showed that the concentration of formate had a stronger correlation with samples distribution.

The results of correlation between environmental factors and microbial community demonstrated that *Acetobacterium* and *Clostridium_sensu_stricto_1* had a positive correlation with acetate and had a negative correlation with temperature. It revealed that the relative lower temperature was conducive to the growth of homoacetogens. Besides, the *Desulfovibrio* had a strong positive correlation with acetate, which might result from that *Desulfovibrio* could degrade lactate to acetate [50]. Unlike *Clostridium_sensu_stricto_1,* the hydrogenotrophic methanogens *Methanobacterium* showed a positive correlation with temperature and formate. Notably, negative correlation was presented between *Methanobacterium* and *Acetobacterium* as well as acidogenic

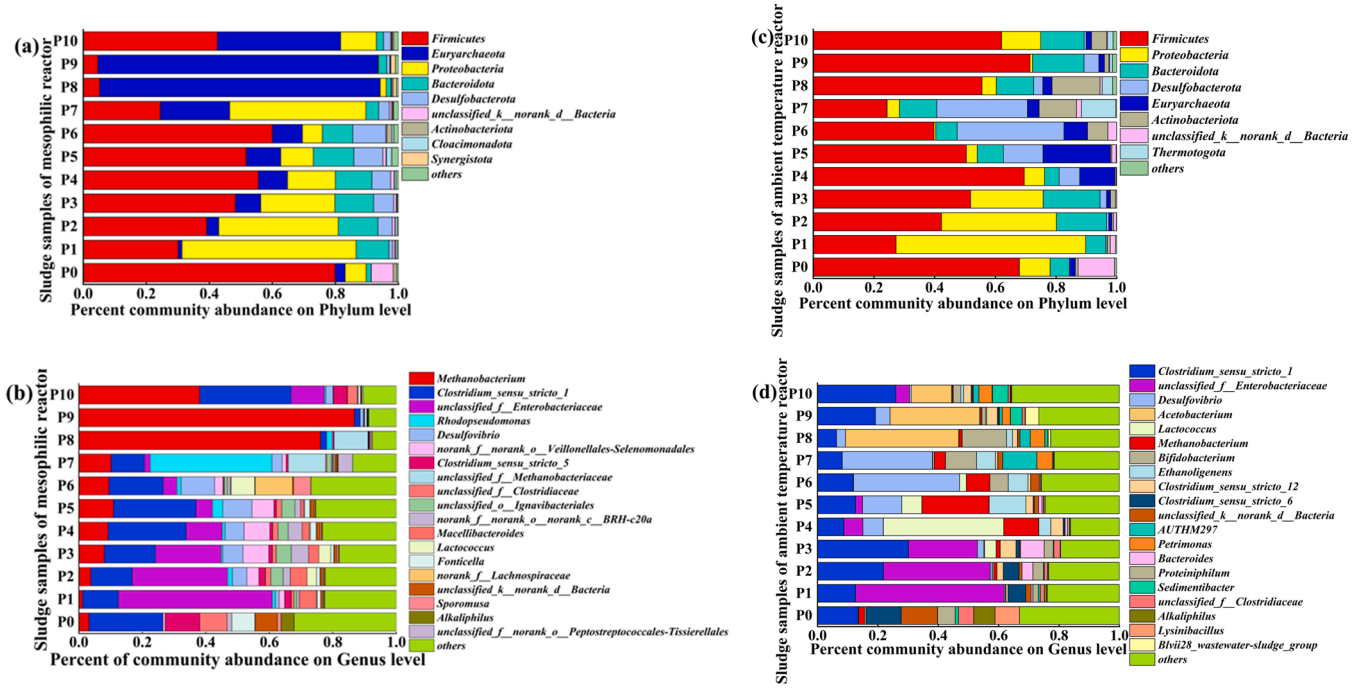

Fig. 6. Diversity and relative abundance of archaea and bacteria on phylum and genus level: mesophilic reactor on phylum level (a); mesophilic reactor on genus level (b); ambient reactor on phylum level (c); ambient reactor on genus level (d).

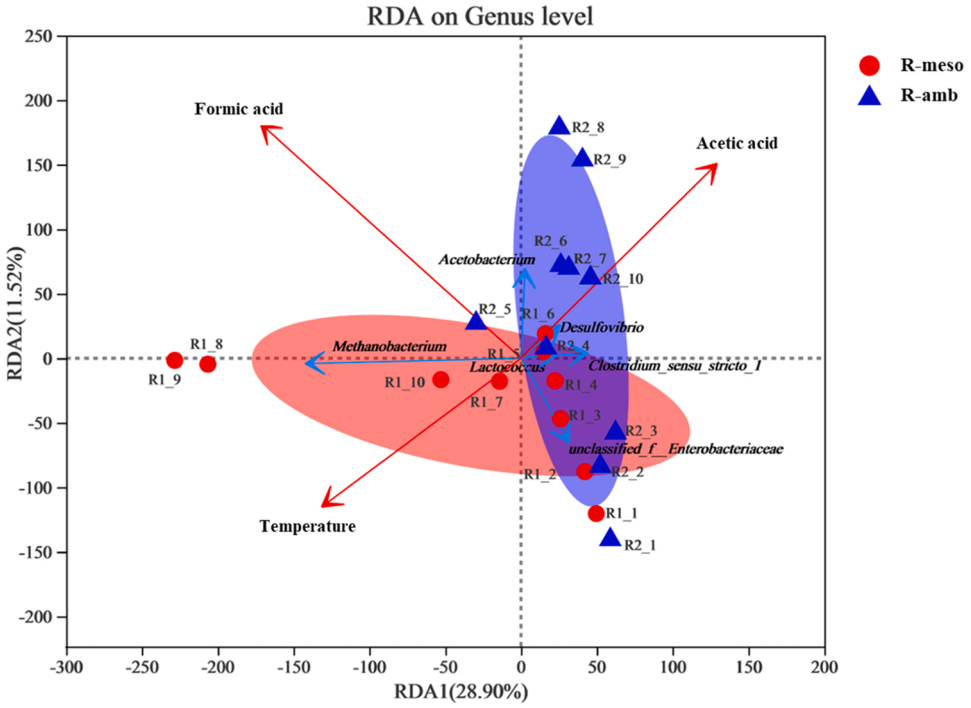

Fig. 7. Redundancy analysis (RDA) of environmental factors, sludge samples and microbial distribution; the red arrows represented the environmental factors of temperature, formate and acetate, the length represented the correlation level.

microorganisms including *Clostridium_sensu_stricto_1* and *Lactococcus* with formate addition. The mesophilic condition was beneficial to the growth of *Methanobacterium* and further competed on the formate consumption with *Acetobacterium*.

The results of RDA indicated that the enrichment of homoacetogens was highly influenced by temperature and substrates. In agreement with the microbial community succession presented in Fig. 6 and quantity of homoacetogens showed in Fig. 8, the ambient temperature and formate content were the most important factors for enriching homoacetogens.

3.2.3. Quantification of expression of formyltetrahydrofolate synthetase (*fhs*) gene

The *fhs* gene in acetyl-CoA pathway of acetogenesis can be used as probe to quantity homoacetogens [55]. The quantitative variations of *fhs* at different phases in R-meso and R-amb are shown in Fig. 8. Generally, the quantity of *fhs* showed a significant increase in the two

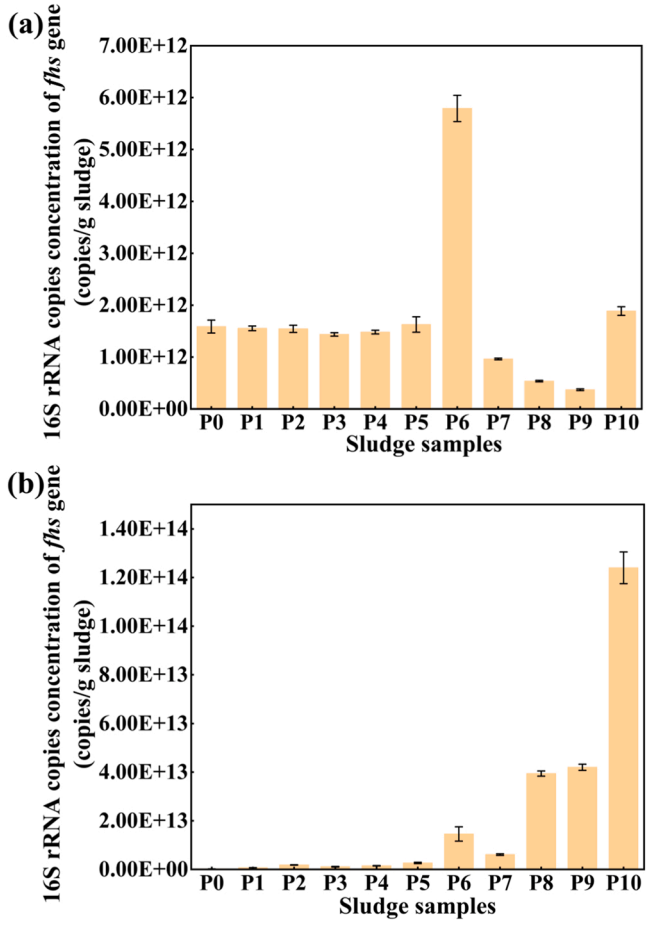

Fig. 8. 16S rRNA gene copies concentration of *fhs* at different phases of CSTR (mesophilic reactor (a), ambient temperature reactor (b)).

reactors during the enrichment operation, while the time of reaching the peak values varied. In R-amb, the 16 S rRNA copies concentration of *fhs* showed a gradually increase and reached the maximum value of 1.24×10^{14} copies/g sludge at P10, was 579.46 times higher than the 2.14×10^{11} copies/g sludge at P0, much higher than the 467.69 times improvement in previous study [19]. These results suggest that homoacetogens in this reactor could grow well with glucose as well as with H_2/CO_2 [33]. However, in R-meso, the absolute abundance of homoacetogens reached the peak (5.79×10^{12} copies/g sludge) at P6 and presented reduced tendency at subsequent P7-P9. It can be explained by the reappearance of hydrogenotrophic methanogens with feeding formate (P7-P9) (Fig. 6b) that competed on the consumption of formate. In addition, *fhs* gene showed higher abundance in R-amb than in R-meso in all phases, thus, ambient temperature was clearly superior in promoting the enrichment of homoacetogens.

3.3. Metabolic pathways and predicted potential functions

To reveal the potential functions in acidogenic and homoacetogenic pathways, relevant functional genes obtained from P3 and P10 periods, were analyzed by PICRUSt2 functional predictive analysis.

3.3.1. Potential pathways of acidogenic fermentation

As shown in Fig. 9a, the acidogenic fermentation of glucose is comprising three main phases including glycolysis, pyruvate metabolism and acetogenesis [56]. Glucose is firstly degraded to pyruvate via glycolysis, and then through pyruvate generate various products including acetate, propionate, butyrate, H_2 and CO_2.

For propionate generation pathway, propionate could be transformed from propanoyl-CoA via propionate CoA-transferase (EC: 2.8.3.1). Besides, propionate could be generated via an optional pathway by phosphate acetyltransferase (EC: 2.3.1.8) and acetate kinase (EC: 2.7.2.1) [56]. Compared to P3, except for the relative abundance of EC: 2.7.2.1 improved at P10, the changes of EC: 2.8.3.1 and EC: 2.3.1.8 presented reduced trend at P10 in ambient temperature system. The reducing abundance of EC: 2.3.1.8 would restrict the transformation of propanoyl-CoA to propanoyl-P and further decreased the production of propionate. Meanwhile, the abundance of EC: 2.8.3.1 increased and the other two decreased at P10 in R-meso. Therefore, as the relative abundance of genes responsible for the propionate generation decreased at P10, corresponding to the reduced proportion of propionate in both reactors as presented in Fig. 3.

As presented in Fig. 9a, the variation tendency of the functional genes relative to the butyrate generation pathway before and after the enrichment was not significant. This was consistent with butyrate being the major VFA_S both before and after enrichment. Some genes such as coding acetyl-CoA C-acetyltransferase (EC: 2.3.1.9) and acetoacetyl-CoA reductase (EC:1.1.1.36) presented an enhancement trend at P10 in R-amb, while exhibited a reduction trend in R-meso. Meanwhile, some functional genes showed an opposite trend in both reactors, such as butyrate kinase (EC: 2.7.2.7) and acetate CoA-transferase (EC: 2.8.3.8). In general, the still higher abundance of functional genes involved in the butyrate generation pathway at P10 could explain the accumulation of butyrate at P10 as Fig. 3 showed.

Overall, acetate was the primary product of most acidogenic pathways. Acetate can be generated directly from pyruvate through the acetyl-CoA pathway as presented in Fig. 9a [56]. In this pathway, some key genes such as pyruvate ferredoxin oxidoreductase alpha subunit (EC: 1.2.7.1) and acetyl-CoA hydrolase (EC: 3.1.2.1), its relative abundance significantly increased at P10 in R-amb. Meanwhile, the mesophilic condition didn't facilitate the enhancement of related genes. This result suggested that the enrichment in R-amb could raise the capacity of acetate generation of fermentation pathway.

3.3.2. Potential pathway of homoacetogenic

Additionally, acetate can be produced via the homoacetogenic pathway. The homoacetogenic pathway and relative abundance of related functional genes were showed in Fig. 9b. In the homoacetogenic pathway, the relative abundance of most functional genes including methylenetetrahydrofolate reductase [NAD(P)H] (EC: 1.5.1.20), methylenetetrahydrofolate dehydrogenase (NADP+) (EC:1.5.1.5), methylenetetrahydrofolate dehydrogenase (NADP+) (EC: 3.5.4.9), acetyl-CoA synthase (EC: 2.3.1.169), 5-methyltetrahydrofolate corrinoid (EC: 2.1.1.258), formate–tetrahydrofolate ligase (EC: 6.3.4.3) and (EC: 2.7.2.1) increased in ambient reactor while decreased in mesophilic system at P10. Among these functional genes, the abundance of EC: 2.3.1.169 and EC: 2.1.1.258 improved prominently at P10 in ambient temperature reactor while almost disappeared in mesophilic reactor. The genes encoding 2.3.1.169 regulating the conversion of 5-Methyl-tetrahydrofolate to Acetyl-CoA, the Acetyl-CoA was the key gene of homoacetogenic pathway [57]. This result accords with the quantities of *fhs* presented in the Fig. 8 that the abundance of *fhs* increased in ambient reactor while reduced in mesophilic reactor at P10. Suggesting that the homoacetogenic pathway in ambient temperature system overcome the adverse influence of hydrogenotrophic methanogens and enhanced after the enrichment. Summarily, the promotion of acetate production at P10 presented in Fig. 3 was mainly ascribed to the enhanced homoacetogenic pathway [32].

4. Conclusion

The homoacetogens were enriched in mesophilic and ambient CSTR respectively by feeding glucose, glucose/formate and formate at different phases. Summarily, the enrichment of homoacetogens could be attained in acidogenic reactors. And ambient temperature was more

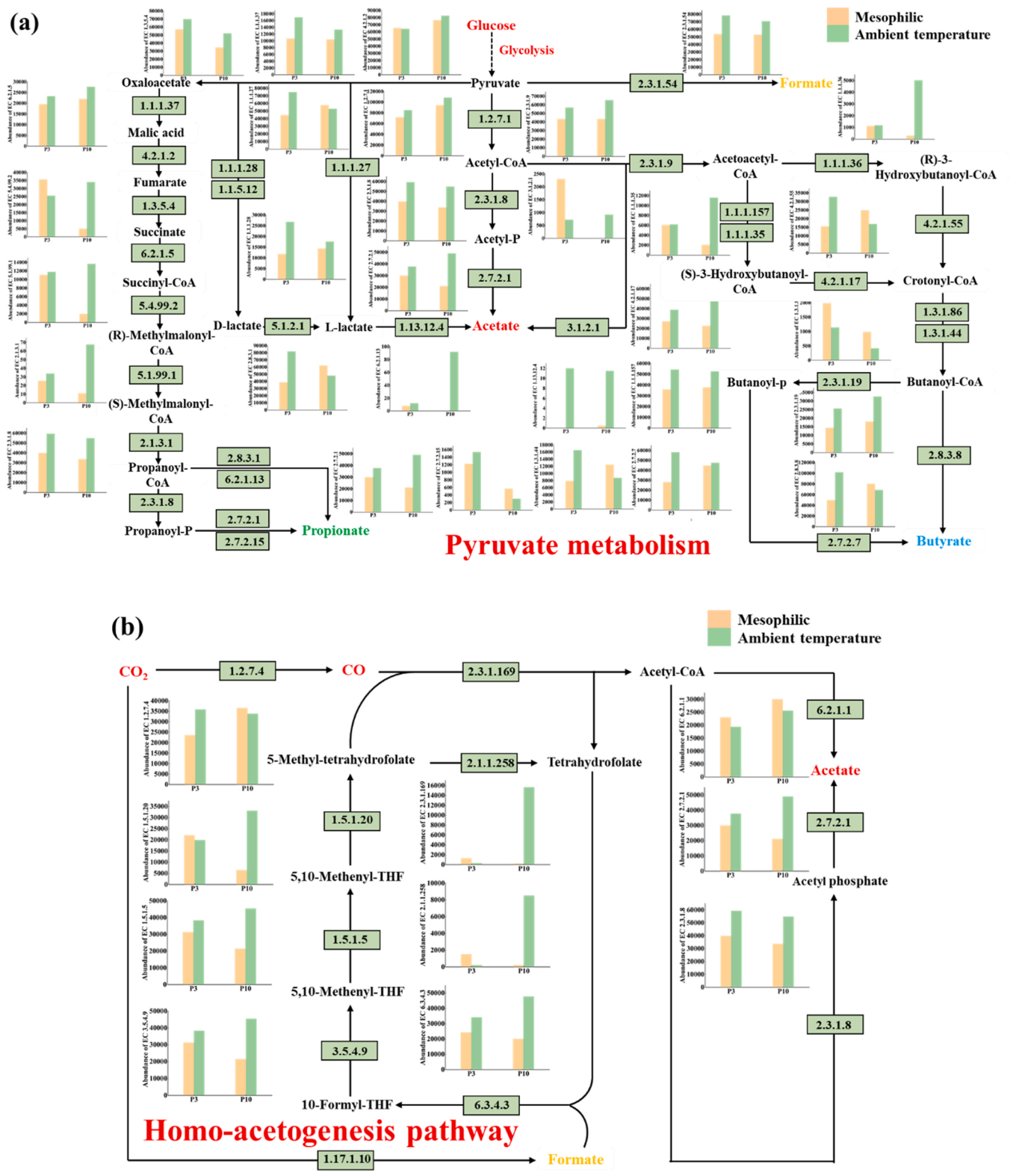

Fig. 9. Variations of relative abundance of functional enzyme-encoding genes involved in pyruvate metabolism pathway (a) and homoacetogenic pathway (b).

conducive to the enrichment of homoacetogens. *fhs*, which representing the abundance of homoacetogens, raised to 1.24×10^{14} copies/g wet sludge at P10 from 2.14×10^{11} copies/g wet sludge at original sludge (P0). Furthermore, enrichment of homoacetogens prompted more carbon flowing to small molecular acid including formate and acetate, which can be utilized by methanogens directly. The genus *Acetobacterium* was the main enriched homoacetogens in this study and had positive correlation with acetate, while had negative correlation with temperature. The enrichment strategy in this work provides novel technique solution and methodological foundation for further study into the solution of accumulation of VFAs in anaerobic digestion system. However, in order to apply this technique solution for combating VFAs accumulation in real practice, further studies, including how to start up the homo-acetogenic dominant acidogenic phase rapidly and how to

improve the carbon flow rate to VFAs, are required. In addition, it is quite necessary to investigate how the homoacetogens enriched CSTR system operate when treating real waste(water), which is our next step study.

CRediT authorship contribution statement

Zunjing Lv: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Xiaofang Pan:** Conceptualization, Resources, Funding acquisition, Formal analysis, Validation, Writing – review & editing. **Zhi-Long Ye:** Project administration, Supervision. **Irini Angelidaki:** Supervision, Validation. **Nan Lv:** Writing – review & editing, Visualization. **Yanlin Li:** Supervision, Formal analysis. **Guanjing Cai:** Writing – review & editing. **Lifeng Lin:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This research was financially sponsored by the National Natural Science Foundation of China (grant number: 51808525, 22006146), Fujian Provincial Science and Technology Project, Social Development Guiding (Key) Projects (grant number: 2021Y0070), Joint Foundation Projects of Key Laboratory of Urban Pollutant Conversion, Chinese Academy of Sciences (KLUPC-2022-4) and Xiamen Natural Science Foundation Project (3502Z20227237).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2023.110224.

References

[1] N. Lv, L.X. Zhao, R.M. Wang, J. Ning, X.F. Pan, C.X. Li, G.J. Cai, G.F. Zhu, Novel strategy for relieving acid accumulation by enriching syntrophic associations of syntrophic fatty acid-oxidation bacteria and H_2/formate-scavenging methanogens in anaerobic digestion-sciencedirect, Bioresour. Technol. (2020) 313, https://doi.org/10.1016/j.biortech.2020.123-702.

[2] A. Puupponen, A. Lonkila, A. Savikurki, K. Karttunen, S. Huttunen, A. Ott, Finnish dairy farmers' perceptions of justice in the transition to carbon-neutral farming, J. Rural Stud. 90 (2022) 104–112, https://doi.org/10.1016/j.jrurstud.2022.01.014.

[3] X.F. Pan, N. Lv, G.J. Cai, M.D. Zhou, R.M. Wang, C.X. Li, J. Ning, J.J. Li, Y.L. Li, Z. L. Ye, G.F. Zhu, Carbon- and metal-based mediators modulate anaerobic methanogenesis and phenol removal: focusing on stimulatory and inhibitory mechanism, J. Hazard. Mater. 420 (2021) 126–615, https://doi.org/10.1016/j.jhazmat.2021.126615.

[4] L.D. Shi, Z. Wang, T. Liu, M. Wu, H.P. Zhao, Making good use of methane to remove oxidized contaminants from wastewater, Water Res. 197 (121228) (2021), 117082, https://doi.org/10.1016/j.watres.2021.117082.

[5] R.J. Ni, C.M. Xu, X.Y. Shi, S. Yang, L. Li, X.Y. Peng, L.Y. Song, Acetoclastic methanogenesis pathway stability despite the high microbial taxonomic variability in the transition from acidogenesis to methanogenesis during food waste anaerobic digestion, J. Clean. Prod. (2022) 133–758, https://doi.org/10.1016/j.jclepro.2022.133758.

[6] R.M. Wang, N. Lv, C.X. Li, G.J. Cai, X.F. Pan, Y.L. Li, G.F. Zhu, Novel strategy for enhancing acetic and formic acids generation in acidogenesis of anaerobic digestion via targeted adjusting environmental niches, Water Res. 193 (9) (2021), 116896, https://doi.org/10.1016/j.watres.2021.116-896.

[7] Q.C. Meng, H.B. Liu, H.D. Zhang, S.Y. Xu, E. Lichtfouse, Y.B. Yun, Anaerobic digestion and recycling of kitchen waste: a review, Environ. Chem. Lett. 20 (2022) 1745–1762, https://doi.org/10.1007/s10311-022-01408-x.

[8] T. Mechichi, S. Sayadi, Evaluating process imbalance of anaerobic digestion of olive mill wastewaters, Process Biochem. 40 (1) (2005) 139–145, https://doi.org/10.1016/j.procbio.2003.11.050.

[9] K. Sani, R. Jariyaboon, S. O-Thong, B. Cherslip, P. Kaparaju, Y. Wang, P. Kongjan, Performance of pilot scale two-stage anaerobic co-digestion of waste activated sludge and greasy sludge under uncontrolled mesophilic temperature, Water Res. (2022) 118–736, https://doi.org/10.1016/j.watres.2022.118736.

[10] T. Tian, S. Qiao, C. Yu, J.T. Zhou, Effects of nano-sized MnO_2 on methanogenic propionate and butyrate degradation in anaerobic digestion, J. Hazard. Mater. 364 (15) (2018) 11–18, https://doi.org/10.1016/j.jhazmat.2018.09.081.

[11] S.Y. Xu, R.Q. Han, Y.C. Zhang, C.Q. He, H.B. Liu, Differentiated stimulating effects of activated carbon on methanogenic degradation of acetate, propionate and butyrate, Waste Manag. 76 (2018) 394–403, https://doi.org/10.1016/j.wasman.2018.03.037.

[12] Q.D. Yin, G.X. Wu, Advances in direct interspecies electron transfer and conductive materials: electron flux, organic degradation and microbial interaction, Biotechnol. Adv. 37 (8) (2019), 107443, https://doi.org/10.1016/j.biotechadv.2019.107-443.

[13] G.J. Wang, Y. Li, L. Sheng, Y. Xing, G.H. Liu, G.F. Yao, H.H. Ngo, Q. Li, X.H. Wang, Y.Y. Li, R. Cheng, A review on facilitating bio-wastes degradation and energy recovery efficiencies in anaerobic digestion systems with biochar amendment, Bioresour. Technol. (2020) 314, https://doi.org/10.1016/j.biortech.2020.123-777.

[14] Y. Li, L. Li, Y. Sun, Z. Yuan, Bioaugmentation strategy for enhancing anaerobic digestion of high C/N ratio feedstock with methanogenic enrichment culture, Bioresour. Technol. 261 (2018) 188–195, https://doi.org/10.1016/j.biortech.2018.02.069.

[15] Q.Y. Ban, L.G. Zhang, J.Z. Li, Shift of propionate-oxidizing bacteria with HRT decrease in an UASB reactor containing propionate as a sole carbon source, Appl. Biochem. Biotechnol. 175 (1) (2015) 274–286, https://doi.org/10.1007/s12010-014-1265-8.

[16] P. Wang, H.T. Wang, Y.Q. Qiu, L.H. Ren, B. Jiang, Microbial characteristics in anaerobic digestion process of food waste for methane production-a review, Bioresour. Technol. 248 (2020) 29–36, https://doi.org/10.1016/j.biortech.2021.125-715.

[17] H.L. Drake, A.S. Gossner, S.L. Daniel, Old acetogens, new light, Ann. N. Y. Acad. Sci. 1125 (1) (2010) 100–128, https://doi.org/10.1196/annals.1419.016.

[18] Y. Li, H.P. Fang, T. Noike,) Recent developments in microbiology and biochemistry of homoacetogens and syntrophic acetogens. In: Proceedings of the Seventh International Symposium on Anaerobic Digestion, Oral Paper Preprints, Cape Town, South Africa, 1994, 54e57.

[19] H. Liu, J.S. Shi, X.M. Zhan, L.J. Zhang, B. Fu, H.B. Liu, Selective acetate production with CO_2 sequestration through acetogen-enriched sludge inoculums in anaerobic digestion, Biochem. Eng. J. 121 (2017) 163–170, https://doi.org/10.1016/j.bej.2017.02.008.

[20] Y.X. He, C. Cassarini, F. Marciano, P.N.L. Lens, Homoacetogenesis and solventogenesis from H_2 /CO_2 by granular sludge at 25, 37 and 55 °C, Chemosphere 265 (2021) 128–649, https://doi.org/10.1016/j.chemosphere.2020.128649.

[21] W. Park, S.H. Hyun, S.E. Oh, B.E. Logan, I.S. Kim, Removal of headspace CO_2 increases biological hydrogen production, Environ. Sci. Technol. 39 (12) (2005) 4416–4420, https://doi.org/10.1021/es048569d.

[22] K.W. Xu, H. Liu, G.C. Du, J. Chen, Real-time PCR assays targeting formyltetrahydrofolate synthetase gene to enumerate acetogens in natural and engineered environments, Anaerobe 15 (5) (2009) 204–213, https://doi.org/10.1016/j.Anaerobe.2009.03.005.

[23] P. Tsapekos, M. Alvarado-Morales, I. Angelidaki, H_2 competition between homoacetogenic bacteria and methanogenic archaea during biomethanation from a combined experimental-modelling approach, Environ. Chem. Eng. (2022) 10–1016, https://doi.org/10.1016/j.jece.2022.107-281.

[24] X.M. Wang, X. Bai, Z.F. Li, X.Q. Zhou, S.K. Cheng, J.C. Sun, T. Liu, Evaluation of artificial neural network models for online monitoring of alkalinity in anaerobic co-digestion system, Biochem. Eng. J. 140 (2018) 85–92, https://doi.org/10.1016/j.bej.2018.09.010.

[25] T. Schmidt, P. Harris, S. Lee, B.K. Mccabe, Investigating the impact of seasonal temperature variation on biogas production from covered anaerobic lagoons treating slaughterhouse wastewater using lab scale studies, J. Environ. Chem. Eng. 7 (3) (2019), https://doi.org/10.1016/j.jece.2019.103077.

[26] J. Lier, J. Martin, G. Lettinga, Effect of temperature on the anaerobic thermophilic conversion of volatile fatty acids by dispersed and granular sludge, Water Res. 30 (1) (1996) 199–207, https://doi.org/10.1016/0043-1354(95)00107-V.

[27] O.R. Kotsyurbenko, M.V. Glagolev, A.N. Nozhevnikova, R. Conrad, Competition between homoacetogenic bacteria and methanogenic archaea for hydrogen at low temperature, FEMS Microbiol. Ecol. 38 (2001) 153–159, https://doi.org/10.1016/j.apenergy.2022.119575.

[28] P.L. McCarty, D.P. Smith, Anaerobic wastewater treatment, Environ. Sci. Technol. 20 (12) (1986) 1200–1206, https://doi.org/10.1021/es00154a002.

[29] H.B. Nielsen, H. Uellendahl, B.K. Ahring, Regulation and optimization of the biogas process: propionate as a key parameter, Biomass Bioenergy (2007) 820–830, https://doi.org/10.1016/j.biombioe.2007.04.004.

[30] X.F. Pan, I. Angelidaki, M. Alvarado-Morales, H.G. Liu, Y.H. Liu, X. Huang, G. F. Zhu, Methane production from formate, acetate and H_2 /CO_2: focusing on kinetics and microbial characterization, Bioresour. Technol. 218 (2016) 796–806, https://doi.org/10.1016/j.biortech.2016.07.032.

[31] F.A.M. Bok, C.M. Plugge, A.J.M. Stams, Interspecies electron transfer in methanogenic propionate degrading consortia, Water Res. 38 (6) (2004) 1368–1375, https://doi.org/10.1016/j.waters.2003.11.028.
[32] J. Nolling, G. Breton, M.V. Omelchenko, K.S. Makarova, Q. Zeng, R. Gibson, H. M. Lee, J. Dubois, D. Qiu, J. Hitti, Y.I. Wolf, R.L. Tatusov, F. Sabathe, L. Doucette-Stamm, P. Soucaille, M.J. Daly, G.N. Bennett, E.V. Koonin, D.R. Smith, Genome sequence and comparative analysis of the solvent-producing bacterium Clostridium acetobutylicum, J. Bacteriol. 183 (16) (2001) 4823–4838, https://doi.org/10.1128/JB.183.16.4 823-4838.2001.
[33] G. Luo, D. Karakashev, L. Xie, Q. Zhou, Angelidaki, Long-term effect of inoculum pretreatment on fermentative hydrogen production by repeated batch cultivations: homoacetogenesis and methanogenesis as competitors to hydrogen production, Biotechnol. Bioeng. 108 (2011) 1816–1827, https://doi.org/10.1002/bit.23122.
[34] S. Karekar, R. Stefanini, B. Ahring, Homo-acetogens: their metabolism and competitive relationship with hydrogenotrophic methanogens, Microorganisms (2022) 2076–2607, https://doi.org/10.3390/microorganisms10020397.
[35] T. Wood, The biology, physiology and ecology of termites, in: In: Economic Impact and Control of Social Insects, 1986, Praeger Publishers, Westport, CT, USA, 1986, pp. 1–67.
[36] Y.H. Ahn, R.E. Speece, Elutriated acid fermentation of municipal primary sludge, Water Res. 40 (2006) 2210–2220, https://doi.org/10.1016/j.watres.2006.03.022.
[37] M. Kim, C.Y. Gomec, Y. Ahn, R.E. Speece, Hydrolysis and acidogenesis of particulate organic material in mesophilic and thermophilic anaerobic digestion, Environ. Technol. Lett. 24 (9) (2003) 1183–1190, https://doi.org/10.1080/09593330309385659.
[38] Y.H. Ahn, J.Y. Bae, S.M. Park, K.S. Min, Anaerobic digestion elutriated phased treatment of piggery waste, Water Sci. Technol. 49 (5–6) (2004) 181–189, https://doi.org/10.2166/wst.2004.0752.
[39] B. Haroun, G. Bahreini, M. Zaman, E. Jang, F. Okoye, E. Elbeshbishy, D. Santoro, J. Walton, A. Al-Omari, C. Muller, K. Bell, G. Nakhla, Vacuum-enhanced anaerobic fermentation: Achieving process intensification, thickening and improved hydrolysis and VFA yields in a single treatment step, Water Res. (2022) 118–719, https://doi.org/10.1016/j.watres.2022.118719.
[40] Liu, C.G., Xue, C., Lin, Y.H., Bai, F.W., Redox potential control and applications in microaerobic and anaerobic fermentations. Biotechnol. Adv., 31(2). 257–265. DOI: 10.1016/j.biotechadv.2012.11.005.
[41] N. Lv, G.J. Cai, X.F. Pan, Y.L. Li, R.M. Wang, J.J. Li, C.X. Li, G.F. Zhu, pH and hydraulic retention time regulation for anaerobic fermentation: focus on volatile fatty acids production/distribution, microbial community succession and interactive correlation, Bioresour. Technol. 347 (2022) 126–310, https://doi.org/10.1016/j.biortech.2021.126-310.
[42] Z.H. Zhang, P. Gao, J.Q. Cheng, G.H. Liu, X.Q. Zhang, Y.J. Feng, Enhancing anaerobic digestion and methane production of tetracycline wastewater in EGSB reactor with GAC/NZVI mediator, Water Res. 136 (2018) 54–63, https://doi.org/10.1016/j.watres.2018.02.025.
[43] F. Wang, W.Y. Dong, H.J. Wang, Y., A. Zhao, Z.L. Zhao, J. Huang, T. Zhou, Z.J. Wu, W.T. Li, Enhanced bioremediation of sediment contaminated with polycyclic aromatic hydrocarbons by combined stimulation with sodium acetate/phthalic acid, Chemosphere (2021), 132770, https://doi.org/10.1016/j.chemosphere.2021.132770.
[44] E. Rosenberg, E.F. Delong, S. Lory, E. Stackebrandt, F. Thompson. The Prokaryotes: Firmicutes and Tenericutes, fourth ed., Springer-Verlag, New York, 2014 https://doi.org/10.1007/978-3-642-30120-9.
[45] M. Atasoy, O. Eyice, A. Schnürer, Z. Cetecioglu, Volatile fatty acids production via mixed culture fermentation: revealing the link between pH, inoculum type and bacterial composition, Bioresour. Technol. 292 (2019) 121–889, https://doi.org/10.1016/j.biortech.2019.121889.
[46] Z. Fan, D. Wu, Y. Zhang, J. Li, L. Wang, Carbonate alkalinity and dietary protein levels affected growth performance, intestinal immune responses and intestinal micromicroflora in Songpu mirror carp (Cyprinus carpio songpu), Aquaculture (3) (2021), 737135, https://doi.org/10.1016/j.aquaculture.2021.737135.
[47] X.Z. Yang, M.J. Xu, G.Y. Huang, C. Zhang, Y.Y. Pang, Y.X. Cheng, Effect of dietary L-tryptophan on the survival, immune response and gut microbiota of the Chinese mitten crab, Fish. Shellfish Immunol. (2019) 1007–1017, https://doi.org/10.1016/j.fsi.2018.10.076.
[48] K. Paul, J.O. Nonoh, L. Mikulski, A. Brune, "Methanoplasmatales," thermoplasmatales-related archaea in termite guts and other environments, are the seventh order of methanogens, Appl. Environ. Microbiol. 78 (23) (2012) 8245–8253, https://doi.org/10.1128/AEM.02193-12.
[49] V.B. Lanjekar, N.P. Marathe, Y.S. Shouche, D.R. Ranade, Clostridium punense sp. nov. an obligate anaerobe isolated from healthy human faces, Int. J. Syst. Evolut. Microbiol. 65 (12) (2015) 4749, https://doi.org/10.1099/ijsem.0.000644.
[50] M.S. Donnenberg, Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, Elsevier, 2015, pp. 2503–2517, https://doi.org/10.1016/B978-1-4557-4801-3.00220-4.
[51] G. Muyzer, A.J.M. Stams, The ecology and biotechnology of sulphate-reducing bacteria, Nat. Rev. Microbiol. 108 (2008) 18295–18300, https://doi.org/10.1038/nrmicro1892.
[52] C.S. Neuendorf, G.A. Vignolle, C. Derntl, T. Tomin, K. Novak, R.L. Mach, R. B. Grunberger, S. Pflugl, A quantitative metabolic analysis reveals acetobacterium woodii as a flexible and robust host for formate-based bioproduction-sciencedirect, Metab. Eng. (2021), https://doi.org/10.1016/j.ymben.2021.09.004.
[53] W.E. Balch, S. Schoberth, R.S. Tanner, R.S. Wolfe, Acetobacterium, a new genus of hydrogen-oxidizing, carbon dioxide-reducing, anaerobic bacteria, Int. J. Syst. Evolut. Microbiol. (1977) 27–4-355, https://doi.org/10.1099/00207713-27-4-355.
[54] Oren, A., (2014) The Family Methanobacteriaceae. Springer Berlin Heidelberg. ISBN: 978-3-642-38953-5.
[55] A.B. Leaphart, M.J. Friez, C.R. Lovell, Formyltetrahydrofolate synthetase sequences from salt marsh plant roots reveal a diversity of acetogenic bacteria and other bacterial functional groups, Appl. Environ. Microbiol. (2002), https://doi.org/10.1128/aem.69.1.693-696.2003.
[56] J. Li, A. Li, Y. Li, M. Cai, G. Luo, Y. Wu, Y.C. Tian, L.Q. Xing, Q.X. Zhang, Picrust2 functionally predicts organic compounds degradation and sulfate reduction pathways in an acidogenic bioreactor, Front. Environ. Sci. Eng. 016 (004) (2022), https://doi.org/10.1007/s11783-021-1481-8.
[57] X.F. Pan, L.X. Zhao, C.X. Li, I. Angelidaki, N. Lv, J. Ning, G.J. Cai, G.F. Zhu, Deep insights into the network of acetate metabolism in anaerobic digestion: focusing on syntrophic acetate oxidation and homoacetogenesis, Water Res. 190 (6) (2020) 116–774, https://doi.org/10.1016/j.watres.2020.116-774.