Acclimation of Acid-Tolerant Methanogenic Culture for Bioaugmentation: Strategy Comparison and Microbiome Succession

Changrui Wang, Ying Li,* and Yongming Sun

ABSTRACT: To enrich an acid-tolerant methanogenic culture used as bioaugmented seed under acidic conditions, we operated four semicontinuous digesters under various conditions of pH decline for producing methane at pH 5.0. 16S rRNA amplification was performed to unravel the association between declining pH and microbiome succession. The findings demonstrated that a gradual decrease of pH, at a step size of 0.5, and a prolonged run time at each pH could achieve a suitable microbial culture, in which acetoclastic Methanothrix and hydrogenotrophic Methanolinea represented the dominant methanogens. In contrast, a sharp decline in pH could result in heavy loss of the acetoclastic methanogen Methanothrix, leading to a cessation of methane production. Hydrogenotrophic methanogens exhibited high acid tolerance, and Methanospirillum could thrive despite a sudden low-pH shock. Although Methanolinea required a longer time to enrich, it played a substantial role in methane production under an acidic environment.

1. INTRODUCTION

Anaerobic digestion (AD) is an effective technology for organic waste treatment and bioenergy generation. A large number of AD plants have been established worldwide; however, some exhibit low economic efficiency, which is generally attributed to methane yields because of operational challenges and poor management practices. Hence, various intensification approaches have been applied to enhance the methane production of digesters, such as pretreatments to increase the hydrolysis rate of complex waste; codigestion to adjust the carbon/nitrogen ratio of feedstock; optimization of the reactor configuration and operating parameters to increase the organic loading rate; and dosing with trace elements to stimulate microbial activity, as well as in wastewater treatment to enhance removal efficiency. In recent years, bioaugmentation has also been applied to increase the biogas production of AD; for example, to enhance the hydrolysis rate of complex substrates, alleviate ammonia toxicity, increase the organic loading rate, or recover the AD process from ammonia inhibition.

Furthermore, it is generally known that volatile fatty acid (VFA) accumulation under overloading conditions in AD is a common problem. This accumulation would inhibit methanogenesis and even induce AD process failure. Notably, bioaugmentation has successfully recovered deteriorated digesters from organic overloading. However, a precondition for successful bioaugmentation is the availability of specific microorganisms; for example, these might be purchased or enriched through feeding with a selective medium. In particular, the use of bioaugmentation to restore a failing digestion process caused by VFA acidosis may require an acid-tolerant methanogenic culture for several reasons. (1) VFA accumulation is often accompanied by a decrease in pH. In addition, both high VFA concentrations and pH drop exert a toxic and inhibitory effect on microbial activity, followed by a complete system failure in anaerobic digesters. Thus, a candidate that can survive under low pH conditions is important. (2) Compared to other microorganisms involved in AD (e.g., acidogens and acetogens), methanogens may be hardest to hit by dropping pH as they are highly sensitive to environmental changes; therefore, adding methanogens to increase their population might enhance methanogenesis. (3) Finally, the introduced pure strain may be difficult to grow or exhibit limit activity in the digesters owing to competition with the indigenous microbes; hence, a mixed culture appears to
we focused on the comparison of different digestion performance. In the present study, a methanogenic culture and why different acclimation methods have also been described. Moreover, some researchers found that acetotrophic methanogens were a potential alternative for a competitive seed in bioaugmentation compared to a pure strain. Several pure aceticlastic or acido tolerant methanogens have previously been isolated, of which all were hydrogenotrophic methanogens that grew at a pH of 3.8–6.6. Alternatively, enrichment of acido tolerant H2/CO2-utilizing methanogenic cultures has also been described. Moreover, some researchers established the best-performing acid-tolerant microbial community. Our previous study, an acid-tolerant methanogenic culture was enriched, which could produce methane from propionate as a carbon source at a pH between 4.8 and 5.5. However, researchers rarely explored how to obtain an acid-tolerant methanogen that used a pH of 3.8–6.6. Alternatively, enrichment of acido tolerant H2/CO2-utilizing methanogenic cultures has also been described. Moreover, some researchers found that acetotrophic methanogens were a potential alternative for a competitive seed in bioaugmentation compared to a pure strain.

In our previous study, an acid-tolerant methanogenic culture was enriched, which could produce methane from propionate as a carbon source at a pH between 4.8 and 5.5. However, researchers rarely explored how to obtain an acid-tolerant methanogenic culture and why different acclimation methods result in a distinct digestion performance. In the present study, we focused on the comparison of different acclimation strategies and response of microbial communities to various profiles of pH decline to clarify these questions. The findings of this study are expected to benefit the recovery from overloaded digestion or improve the treatment of acidic wastewater, along with providing information for the enrichment of other special microbial cultures.

### 2. MATERIALS AND METHODS

#### 2.1. Experimental Setup

The inoculum was taken from an anaerobic digester treating crops (Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, China). Prior to use, it was sieved through a 1 mm mesh to remove grit and other solids.

The experiment was carried out in a mesophilic semi-continuous stirred-tank-type reactor with 1.0 L of working volume, which was initially inoculated with the sieved digestate, after which the headspace was flushed with a N2/CO2 gas mixture (80:20 v/v). An identical hydraulic retention time (HRT) of 20 days was maintained by removing 50 mL of reactor content and replacing it with 50 mL of feed once daily under anaerobic conditions.

Propionate was chosen as the sole substrate. The organic loading rate was set at 0.53 g of propionic acid L−1 d−1 during the whole experimental period. The volume of the feed was made up by a nutrient medium. The nutrient medium contained the following [mg L−1]: NH4Cl [400]; (NH4)2HPO4 [80]; and FeCl3·6H2O [55]; and the trace element salts (i.e., CoCl2·6H2O, NiCl2·6H2O, MnCl2·4H2O, CuCl2·2H2O, AlCl3·6H2O, ZnCl2, Na2WO4·2H2O, H3BO3, Na2SeO3, and Na2MoO4·2H2O) [each at 0.5].

#### 2.2. Enrichment Strategy

The daily target pH was set based on the following two considerations: (1) which is the optimum initial pH for start-up and (2) how long the digester should be running at each pH level during the gradually reducing pH process. Therefore, we set the daily target initial pH of the four digesters during each HRT, as shown in Table 1.

The pH in the digester increased after 1 day’s AD. In order to maintain the target pH, every day we measured the pH of the digestate first and then we used HCl to adjust the digestate pH to the target pH.

As shown in Table 1, the four digesters proceeded through a variety of different pH values until the target pH had decreased to 6.0 between day 160 and day 280. The daily initial pH of reactor 1 (R1) was set at 6.5 during the first eight HRTs, whereas the other three reactors were started at pH 7.0. The pH values of both R2 and R3 were reduced by 0.5 in each subsequent HRT (days 1–4, reactors 1–4).

#### 2.3. Analytical Methods for Digestion Performance

pH was determined using a FE28-Standard meter (Mettler-Toledo, Zürich, Switzerland) with a glass electrode calibrated in buffers at pH 7.0 and 4.0. VFAs were quantified using an e2698HPLC system (Waters, Milford, CT) equipped with a column (Bio-Rad, Hercules, CA) at 50 °C with 0.5 mM H2SO4 as the mobile phase at a flow rate of 0.5 mL min−1. Methane (CH4) and carbon dioxide (CO2) contents were analyzed using a GC-2014 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a thermal conductivity detector (120 °C) and Porapak Q column (70 °C); argon was used as the carrier gas (20 mL min−1).

#### 2.4. 16S rRNA Gene Amplification

DNA extraction was performed using the fast DNA spin kit for soil (QBiogene, Santa Ana, CA) according to the manufacturer’s instructions. DNA quality was assessed using 1% agarose gel electrophoresis, and DNA concentrations were determined using a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA). The extracted DNA sample was then handled according to the protocol of the genomic DNA sample preparation kit (Illumina, San Diego, CA). First, DNA fragmentation was performed using a Covaris S2 Ultrasonicator (Woburn, MA), and then the DNA fragments were processed by end reparation, A-tailing, adapter ligation, and DNA size selection. PCR and product purification were based on Illumina

---

**Table 1. Daily Target pH of the Four Reactors during Each HRT**

| HRT period (d) | 0–20 | 21–40 | 41–60 | 61–80 | 81–100 | 101–120 | 121–140 | 141–160 | 161–280 | 281–300 | 301–320 |
|---------------|------|-------|-------|-------|--------|--------|--------|--------|--------|--------|--------|
| R1            | 6.5  | 6.5   | 6.5   | 6.5   | 6.5    | 6.5    | 6.5    | 6.5    | 6.0    | 5.5    | 5.0    |
| R2            | 7.0  | 7.0   | 7.0   | 7.0   | 6.5    | 6.5    | 6.0    | 6.0    | 6.0    | 5.5    | 5.0    |
| R3            | 7.0  | 7.0   | 7.0   | 6.5   | 6.5    | 6.5    | 6.0    | 6.0    | 6.0    | 5.5    | 5.0    |
| R4            | 7.0  | 7.0   | 7.0   | 7.0   | 7.0    | 7.0    | 6.0    | 6.0    | 6.0    | 5.5    | 5.0    |

*R1—4, reactors 1–4.*
MiSeq2x300 instructions. For Archaea, the 16S rRNA genes were amplified through three rounds of PCR. The primers for the first round were 340F (5′-CCC TAY GGG GYG CAS CAG-3′) and 1000R (5′-GGC CAT GCA CYW CYT CTC-3′). The PCR products were then used as templates for a second PCR with primers 349F (5′-CCC TAC ACG ACG CTC TTC CGA TCT G (barcode) GYG CAS CAG KCG MGA AW-3′) and 806R (5′-GAC TGG AGT TCC TTG GCA CCC GAG AAT TCC AGA CTA CHV GGG TAT CTA ATC C-3′), followed by a third round of PCR with Illumina nested primers. Bacteria 16S rRNA genes were amplified through two rounds of PCR. The primers for the first round were 341F (5′-CCC TAC ACG CTC TTC CGA TCT G (barcode) CCT ACG GGN GGC WGC AG-3′) and 805R (5′-GAC TGG AGT TCC TTG GCA CCC GAG AAT TCC AGA CTA CHV GGG TAT CTA ATC C-3′), and the PCR products were then used as templates for a second PCR with Illumina nested primers. Prior to sequencing, PCR products of different samples were normalized in equimolar amounts in the final mixture, which was used to construct the PCR amplicon libraries. Sequencing was carried out on an Illumina HiSeq 2000. The obtained sequences were phylogenetically allocated down to the phylum, class, and genus level using the RDP classifier (http://rdp.cme.msu.edu/misc/resources.jsp). The 16S rRNA gene amplification data were deposited in the NCBI Sequence Read Archive database with accession numbers SRR6702441−6702452. To define the relative abundance of a given phylogenetic group, the number of sequences affiliated to that group was divided by the total number of obtained sequences. The results were used for the analysis and comparison of microbial community structure differences. QIIME (http://qiime.org/) was used for network

Figure 1. AD performance in R1, R2, R3, and R4 with different acclimation strategies (the initial pH represents the initial daily pH after adjustment). [VBP = biogas production (L) / time (d) / reactor working volume (L)]

Figure 2. Changes in VFA concentration and pH in R1, R2, R3, and R4.
analysis. Species with abundance >1% were used for network analysis. The network layout was generated using the R language igraph package (https://igraph.org/r/), with significant contact (weight > 100) nodes.

3. RESULTS AND DISCUSSION

3.1. Digestion Performance. Figure 1 shows the volumetric biogas production (VBP) and methane percentage in the four bioreactors. We found that biogas production varied according to the bioreactor. For R1, the daily initial pH was continually adjusted to 6.5 during the first eight retention times (day 0–160); correspondingly, VBP fluctuated between 0.21 and 0.32 L L⁻¹ d⁻¹, and the average volatile solid methane production (VSMP) (methane production per gram VS per day) was 0.28 L g⁻¹ d⁻¹. In addition, both biogas production and methane content of R1 increased slightly after running for 100 days, which may have occurred because of the degradation of the propionate concentration (Figure 2). When the daily initial pH declined to 5.0, R1 could still produce methane with the average VSMP of 0.42 L g⁻¹ d⁻¹, which was approximately 73% of the theoretical methane production, indicating that the digester was gradually adapted to the acidic environment with a pH of 5.0. Both R2 and R3 were able to operate at the pH of 6.0; however, they were prone to failure when the pH was adjusted to 5.5 and even stopped producing biogas upon the lower pH shock of 5.0 (Figure 1). R4 was directly exposed to a daily initial pH of 6.0 from 7.0 on day 161 without running at a pH of 6.5; as a result, biogas production in the following two HRTs dropped markedly, suggesting that the microbial community could not resist such a pH shock. Methane content during the stable states in the four reactors did not significantly differ, being between 50 and 68%.

As shown in Figure 2, after daily fermentation, the pH of R1 rose by 0.5–0.8 compared to the initial target pH. The time-course pH profiles in the other three digesters followed the same trend when the daily initial pH was above 6.0. However, once the daily initial pH was set at 5.5, the increase of pH in R2 and R3 was smaller compared to that of R1. During the last HRT, the pH in R2 and R3 rose only minimally at the end of the operation once the pH was adjusted to 5.0. In addition, the propionate concentration in both reactors increased sharply during the later stage (Figure 2), which was responsible for a significant drop in methane production (Figure 1). The performance of R4 was even worse, with substantial propionate accumulation being observed once the daily initial pH decreased from 7.0 to 6.0. After running at a pH of 6.0 for 16 days, the propionate concentration in R4 reached up to 6.9 g L⁻¹, suggesting that propionate degradation is closely associated with sudden pH decline. Similarly, a previous study also found that a low pH (below 6.5) inhibited propionate degradation in an up-flow anaerobic sludge blanket reactor using propionate as the sole carbon source.

It is obvious from Figures 1 and 2 that R1 showed the best performance, followed by R2 and R3, whereas R4 performed worst. The different capability of biogas production among the four reactors could be ascribed to a variety of pH decline processes. R2 was operating in each pH phase (7.0 and 6.5) for four HRTs, which was longer compared to the three HRTs of R3. This may be the main factor contributing to the better performance of R2 than R3. R4 was directly exposed to pH 6.0 without running at 6.5. Apparently, decreasing pH by 1.0 led to poor methane production at a pH of 6.0 in R4. R1 led the AD during the last HRT with a pH of 5.0, indicating that operating for a longer time at pH 6.5 might be better for the tolerance of lower pH conditions. Moreover, the pH of start-up phase might be an important factor.

3.2. Shift of the Methanogenic Community in Different Digesters. Obvious differences in digestion performance among the four digesters were observed until the daily initial pH was decreased to 6.0. Therefore, the archaeal community was compared when the pH was below 6.0. Specimens were taken from R1, R2, and R3 on day 280 (pH 6.0) and day 320 (pH 5.0). R4 failed when the pH was below 6.0, so specimens of G4 were taken the day 160 (pH 7.0) and day 180 (pH 6.0). Based on the results of 16S rRNA gene amplification, we identified 11 archaeal genera that were capable of methane production, as shown in Figure 3. The results clearly illustrate that at pH 6.0, the abundance of Methanotrichia, an acetoclastic methanogen, was much higher in R1, with 60.53% (15,362 reads), compared with 13.44% (3231 reads) in R4. In contrast, the hydrogenotrophic methanogen Methanospirillum become the absolute majority methanogen in R4 with a relative abundance of 84.31% (20,262 reads). This increase in abundance of hydrogenotrophic methanogens in stressed conditions was also reported previously. For R4, with the shock of pH 6.0, the relative abundance of Methanotrichia decreased markedly from 39.10 to 13.44%. Therefore, the poor performance in R4 might mainly derive from a significant reduction of Methanotrichia.

In addition, the structure of the methanogenic community in R2 was similar to that of R1, but fewer Methanospirillum members were present at pH 5.0 in R2 than in R1. Compared to that in R1 and R2, the abundance of Methanolinea (19.35%) in R3 was relatively lower at pH 5.0.

Based on the response of the functional microbes in the four digesters to low pH, it could be conjectured that a sudden shock of low pH had a significant impact on acetoclastic methanogens. In contrast, hydrogenotrophic methanogens exhibited high acid tolerance. Moreover, prolonging the acclimation period and gradually decreasing the pH appears to be beneficial for improving the adaptability of acetoclastic methanogens to acidic conditions.

3.3. Microbial Network of Methanogenic Culture during the Acid Adaptation Process. As it yielded the best methane production under acidic conditions, the culture from R1 was selected from the four digesters for further bioaugmentation. Prior to its application, it was essential to comprehensively understand the microbial community structure and its dynamic change with pH; therefore, a microbial

![Figure 3. Response of main archaeal genera associated with methane generation under acidic conditions (the size of each sphere indicates the read number of the 16S rRNA gene; numbers 5, 6, and 7 following the hyphen represent the pH value).](http://pubs.acs.org/content/acsomega/10.1021/acsomega.9b03783)
network was constructed based on the samples taken under various pH conditions (pH 6.5, 6.0, 5.5, and 5.0), as shown in Figure 4. Overall, 732 genera were detected in all samples; Figure 4 only shows the main genera with proportions >1%.

In detail, the biggest pie represents genus *Rhodobacter*, indicating that it had a high occurrence rate during the whole acclimation period. Moreover, the color partition of *Rhodobacter* in the pie indicates that its abundance was increasing along with a dropping pH, with a rise from 0.40% at pH 6.5 (light blue) to 44.07% at pH 5.0 (yellow). In particular, some species affiliated to genus *Rhodobacter* have been commonly reported as supporting photo fermentative hydrogen production. These enable the production of hydrogen from malate and glutamate, in addition to various VFAs such as acetic acid and lactate. If *Rhodobacter* produced hydrogen in R1, the hydrogen may have been immediately scavenged by hydrogen-consuming microbes, such as hydrogenotrophic methanogens, as no hydrogen was detected in the biogas content.

The second biggest pie in Figure 4 represents *Methanothrix*, which is the dominant acetotrophic methanogen in the reactor. Its occurrence decreased with the dropping pH from the maximum read numbers of 15,857 at pH 6.5 to 8944 at pH 5.0. The feasible pH range for acetotrophic methanogens is reportedly between 6.6 and 7.3, and *Methanothrix* therefore decreased once the pH was lower than 6.5 in R1.

In addition, several functional members also occupied the center positions in the network, such as *Syntrophobacter*, *Syntrophomonas*, *Smithella*, and *Pelotomaculum*. These were closely correlated to the degradation of propionate. The proportion of *Syntrophobacter* rose from 1.03% in the inoculum to 8.83% on day 280. Even though the pH was lower at 5.5 and 5.0 during the last two HRTs, *Syntrophobacter* still maintained its abundance above 8.25%, leading to a relatively high ability of propionate conversion in R1. The high occurrence of *Syntrophobacter* has been observed in previous studies.

Although the pure strain of *Syntrophobacter* has rarely been reported to grow at pH 5.0, *Syntrophobacter* appeared to outnumber other propionate-oxidizing bacteria in R1, reinforcing the importance of this genus to overall propionate degradation under acidic conditions.

4. CONCLUSIONS

In conclusion, an acid-tolerant methanogenic culture, producing methane from propionate at pH 5.0, was obtained by a method of stepwise pH reduction, dominated by acetoclastic *Methanothrix* and hydrogenotrophic *Methanolinea*. This culture might be considered as a candidate for bioaugmentation of overloaded digestion recovery and acidic wastewater treatment. Different acclimation strategies exhibited various microbial community compositions: a sudden shock of low pH could enrich a hydrogenotrophic methanogen-dominant culture, whereas prolonged and gradual decrease of pH resulted in the enrichment of mixed acetotrophic and hydrogenotrophic methanogens. These results together indicate the feasibility of generating a desired enrichment by designing appropriate acclimation processes.
AUTHOR INFORMATION

Corresponding Author
Ying Li — Laboratory of Biomass Bio-Chemical Conversion, Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou 510640, PR China; orcid.org/0000-0002-0625-2554; Email: liying@gsc.ge.ac.cn

Authors
Changrui Wang — College of Energy and Power Engineering, Lanzhou University of Technology, Lanzhou 730050, China; Laboratory of Biomass Bio-Chemical Conversion, Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou 510640, PR China; Key Laboratory of Complementary Energy System of Biomass and Solar Energy, Lanzhou 730050, Gansu Province, China
Yongming Sun — Laboratory of Biomass Bio-Chemical Conversion, Guangzhou Institute of Energy Conversion and Key Laboratory of Renewable Energy, Chinese Academy of Sciences, Guangzhou 510640, PR China; Guangdong Provincial Key Laboratory of New and Renewable Energy Research and Development, Guangzhou 510640, PR China

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.9b03783

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (grant number 51708538), the Strategic Priority Research Program of the Chinese Academy of Sciences (no. XDA21050400), and the Youth Innovation Promotion Association, CAS.

ABBREVIATIONS

AD, anaerobic digestion; HRT, hydraulic retention time; VBP, volumetric biogas production; VFA, volatile fatty acid; VSMP, volatile solid methane production

REFERENCES

(1) Chen, J. L.; Ortiz, R.; Steele, T. W. J.; Stuckey, D. C. Toxicants inhibiting anaerobic digestion: A review. Biotechnol. Adv. 2014, 32, 1523–1534.
(2) Dev, S.; Saha, S.; Kurade, M. B.; Salama, E. S.; El-Dalatony, M. M.; Ha, G. S.; Chang, S. W.; Jeon, B. H. Perspective on anaerobic digestion for biomethanation in cold environments. Renew. Sustain. Energy Rev. 2019, 103, 85–95.
(3) Kang, X.; Sun, Y.; Li, L.; Kong, X.; Yuan, Z. Improving methane production from anaerobic digestion of Pennisetum Hybrid by alkaline pretreatment. Bioresour. Technol. 2018, 255, 205–212.
(4) Mata-Alvarez, J.; Dosta, J.; Romero-Güiza, M. S.; Fonoll, X.; Peces, M.; Astals, S. A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renew. Sustain. Energy Rev. 2014, 36, 412–427.
(5) Jain, S.; Jain, S.; Wolf, I. T.; Lee, J.; Tong, Y. W. A comprehensive review on operating parameters and different pretreatment methodologies for anaerobic digestion of municipal solid waste. Renew. Sustain. Energy Rev. 2015, 52, 142–154.
(6) Zhang, W.; Wu, S.; Guo, J.; Zhou, J.; Dong, R. Performance and kinetic evaluation of semi-continuously fed anaerobic digesters treating food waste: Role of trace elements. Bioresour. Technol. 2015, 178, 297–305.
(7) Herrero, M.; Stuckey, D. C. Bioaugmentation and its application in wastewater treatment: A review. Chemosphere 2015, 140, 119–128.
(8) Cycoń, M.; Mrozik, A.; Piotrowska-Setge, Z. Bioaugmentation as a strategy for the remediation of pesticide-polluted soil: A review. Chemosphere 2017, 172, 52–71.
(9) Lü, F.; Ji, J.; Shao, L.; He, P. Bacterial bioaugmentation for improving methane and hydrogen production from microalgae. Biotechnol. Biofuels 2013, 6, 92.
(10) Fotidis, I. A.; Wang, H.; Fiedel, N. R.; Luo, G.; Karakash, D. B.; Angelidaki, I. Bioaugmentation as a solution to increase methane production from an ammonia-rich substrate. Environ. Sci. Technol. 2014, 48, 7669–7676.
(11) Li, Y.; Zhang, Y.; Sun, Y.; Wu, S.; Kong, X.; Yuan, Z.; Dong, R. The performance efficiency of bioaugmentation to prevent anaerobic digestion failure from ammonia and propionate inhibition. Bioresour. Technol. 2017, 231, 94–100.
(12) Li, Y.; Li, L.; Sun, Y.; Yuan, Z. Bioaugmentation strategy for enhancing anaerobic digestion of high C/N ratio feedstock with methanogenic enrichment culture. Bioresour. Technol. 2018, 261, 188–195.
(13) Nielsen, H. B.; Angelidaki, I. Strategies for optimizing recovery of the biogas process following ammonia inhibition. Bioresour. Technol. 2008, 99, 7995–8001.
(14) Li, Y.; Yang, G.; Li, L.; Sun, Y. Bioaugmentation for overloaded anaerobic digestion recovery with acid-tolerant methanogenic enrichment. Waste Manag. 2018, 79, 744–751.
(15) Tale, V. P.; Maki, J. S.; Zitomer, D. H. Bioaugmentation of overloaded anaerobic digesters restores function and aerobic community. Water Res. 2015, 70, 138–147.
(16) De Vrieze, J.; Hennebel, T.; Boon, N.; Verstraate, W. Methanosarcina: The rediscovered methanogen for heavy duty biomethanation. Bioresour. Technol. 2012, 112, 1–9.
(17) Cadillo-Quiroz, H.; Yavitt, J. B.; Zinder, S. H. Methanosphaerula palustris gen. nov., sp nov., a hydrogenotrophic methanogen isolated from a minerotrophic fen peatland. Int. J. Syst. Evol. Microbiol. 2009, 59, 928–935.
(18) Kotsyurbenko, O. R.; Friedrich, M. W.; Simkova, M. V.; Nozhevnikova, A. N.; Golyswyn, P. N.; Timmis, K. N.; Conrad, R. Shift from acetoclastic to H2-dependent methanogenesis in a West Siberian peat bog at low pH values and isolation of an acidophilic Methanobacterium strain. Appl. Environ. Microbiol. 2007, 73, 2344–2348.
(19) Bräuer, S. L.; Yashiro, E.; Ueno, N. G.; Yavitt, J. B.; Zinder, S. H. Characterization of acid-tolerant H2/CO2-utilizing methanogenic enrichment cultures from an acidic peat bog in New York State. FEMS Microbiol. Ecol. 2006, 57, 206–216.
(20) Shin, H. C.; Ju, D.-H.; Jeon, B. S.; Choi, O.; Kim, H. W.; Um, Y. J.; Lee, D.-H.; Sang, B.-I. Analysis of the microbial community in an acidic hollow-fiber membrane biofilm reactor (HF-MBR) used for the biological conversion of carbon dioxide to methane. PLoS One 2015, 10, No. e0144999.
(21) Li, Y.; Sun, Y.; Li, L.; Yuan, Z. Acclimation of acid-tolerant methanogenic propionate-utilizing culture and microbial community dissecting. Bioresour. Technol. 2018, 250, 117–123.
(22) Li, Y.; Sun, Y.; Yang, G.; Hu, K.; Lv, P.; Li, L. Vertical distribution of microbial community and metabolic pathway in a methanogenic propionate degradation bioreactor. Bioresour. Technol. 2017, 245, 1029–1029.
(23) Zhang, L.; Ban, Q.; Li, J.; Jha, A. K. Response of syntrophic propionate degradation to pH decrease and microbial community shifts in an UASB reactor. J. Microbiol. Biotechnol. 2016, 26, 1409–1419.
(24) Angenent, L. T.; Sung, S.; Raskin, L. Methanogenic population dynamics during startup of a full-scale anaerobic sequencing batch reactor treating swine waste. Water Res. 2002, 36, 4648–4654.
(25) Mirza, S.; Qazi, J.; Zhao, Q.; Chen, S. Photo-biohydrogen production potential of Rhodobacter capsulatus-PK from wheat straw. Biotechnol. Biofuels 2013, 6, 144.
(26) Basak, N.; Das, D. Photofermentative hydrogen production using purple non-sulfur bacteria Rhodobacter sphaeroides OU001 in an annular photobioreactor: A case study. Biomass Bioenergy 2009, 33, 911−919.
(27) Kim, D.-H.; Son, H.; Kim, M.-S. Effect of substrate concentration on continuous photo-fermentative hydrogen production from lactate using Rhodobacter sphaeroides. Int. J. Hydrogen Energy 2012, 37, 15483−15488.
(28) Pandey, A.; Dolly, S.; Semwal, D.; Pandey, A. Effect of pH on optimization of photofermentative hydrogen production by co-culture of Rhodobacter sphaeroides-NMBL-02 and Bacillus firmus-NMBL-03, Cell. Cell. Mol. Biol. 2017, 63, 68−72.
(29) Demirel, B.; Scherer, P. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: A review. Rev. Environ. Sci. Bio/Technol. 2008, 7, 173−190.