# Effect of bioaugmentation on start-up phase of anaerobic digestion at high organic loading rate

Xingyu Cheng[1,2], Guoxiang Zheng[3], Zhiyuan Hu[2], Miao Yan[2,4*], Feng Zhen[2], Ying Li[1,2], Jiachen Li[2,3], Taili Dong[4], Li Chen[4], Yongming Sun[1*]

(1. *School of Energy Science and Engineering, University of Science and Technology of China, Guangzhou 510640, China*;
2. *Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou 510640, China*;
3. *College of Engineering, Northeast Agricultural University, Harbin 150030, China*;
4. *Shandong Minhe Biotechnology Co., Ltd, Yantai 265600, Shandong, China*)

**Abstract:** In order to enhance the start-up of anaerobic digestion (AD), the propionate-degrading methanogenic cultures were introduced to AD of food waste at a high organic loading rate (OLR) of 3.0 g VS/L·d in this study, and the efficiency of different bioaugmentation strategies were investigated. The results demonstrated that bioaugmentation significantly improved the start-up efficiency and enhanced the methane production. Specifically, higher dosage and frequency of bioaugmentation had a positive effect on the performance of the AD reactors. Among three bioaugmented reactors, the reactor with a bioaugmentation strategy of 0.675 g VS/L of bioaugmentation seed added every 5 d during the first hydraulic retention time (HRT) performed the best and remained relatively stable for the next three HRTs without bioaugmentation. The 16S rRNA gene sequencing analysis revealed that *Methanothrix* predominated in bioaugmented reactors. A large proportion of *Methanothrix* accompanied by a small proportion of *Methanospirillum* played a key role in volatile fatty acid degradation and contributed to the successful start-up and long-term stability of AD at a high OLR. These findings suggest that bioaugmentation with methangenic consortium is a promising strategy to boost the AD process at high OLRs and achieve higher treatment capacity of food waste.

**Keywords:** high organic loading rate, anaerobic digestion, bioaugmentation, propionate degradation, *Methanothrix*
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## 1 Introduction

Improper disposal of up to 33% of food waste (FW) annually worldwide (equivalent to approximately $1.3\times10^9$ t) presents a significant environmental, economic, and human health challenge[1-3]. Traditional methods of organic waste treatment, such as landfills, incineration, composting, and other technologies, often result in greenhouse gas emissions ($CH_4$, $CO_2$) or environmental pollution that is not consistent with sustainable development goals[4-6]. Anaerobic digestion (AD) presents an efficient and eco-friendly solution for disposing of FW while also producing renewable energy in the form of biomethane[7].

FW typically contains a high percentage of volatile solids (10%-30% fresh matter) and is easily degradable, resulting in rapid degradation, acidification, and the production of significant amounts of volatile fatty acids (VFAs) during AD process at high organic loading rate (OLR)[8]. High acidity levels have been known to inhibit methanogen activity, leading to decreased methane yields and system instability[9]. Therefore, AD systems usually start with a low OLR (0.5 g VS/L·d) and gradually increase to a higher OLR to avoid acid accumulation[2]. However, this approach is time-consuming and fails to achieve optimal methane yields during the start-up phase. Thus, effective strategies for starting and maintaining an AD process for FW at high OLRs are necessary.

Various strategies have been proposed to improve the start-up phase of AD. These include using acclimated inoculum to accelerate the process[10], supplying trace elements to increase enzyme content and microbial activity at an initial OLR of 2.0 g VS/L·d[11], adding biochar to improve direct electron transfer between microorganisms to start-up AD at an OLR of 1.5 g VS/ L·d[12], and co-digestion of wastewater sludge with dairy manure to balance the C/N ratio of digestion system[13,14]. The main mechanism behind these methods is to improve the density and activity of functional microbes in the AD system. Bioaugmentation is a promising strategy to promote specific microbial functions[15]. For instance, reactors bioaugmented with a mixed strain of *Methanosarcina barkeri* or *Syntrophaceticu schinkii* with *Methanobrevibacter smithii* showed a 35% increase in methane production[16]. Tian et al.[17] discovered that the bioaugmentation inoculum composed of

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**Biographies: Xingyu Cheng**, PhD candidate, research interest: biochemical utilization and conversion of biomass, Email:chengxy@ms.giec.ac.cn; **Guoxiang Zheng**, professor, research interest: utilization and conversion of biomass, Email: zgx720331@126.com; **Zhiyuan Hu**, MS, research interest: biochemical utilization and conversion of biomass, Email: 1093325239@qq.com; **Feng Zhen**, Senior Engineer, research interest: anaerobic treatment of solid waste, Email: zhenfeng@ms.giec.ac.cn; **Ying Li**, Research fellow, research interest: biochemical utilization and conversion of biomass, Email: liying@ms.giec.ac.cn; **Jiachen Li**, MS, research interest: biochemical utilization and conversion of biomass, Email: l970255188@126.com; **Taili Dong**, MS, research interest: anaerobic digestion, Email: dongtaili@126.com; **Chen Li**, MS, research interest: biochemicalutilizationandconversionofbiomass,Email:chenlichenli2011@163.com.
***Corresponding author: Miao Yan**, PhD, research interest: biochemical utilization and conversion of biomass, No. 2. Wushan Energy Road, Tianhe District, Guangzhou, Tel: +86-15930609301, Email: yanmiao@ms.giec.ac.cn; **Yongming Sun**, Research fellow, research interest: biochemical utilization and conversion of biomass, No. 2. Wushan Energy Road, Tianhe District, Guangzhou, Tel: +86-13288699886, Email: sunym@ms.giec.ac.cn.

hydrogenotrophic methanogen *Methanoculleus thermophilus* stimulated the growth of syntrophic acetate oxidizing bacterium *Thermacetogenium phaeum*, which led to an increase of methane yield by 11%-13% and a decrease of volatile fatty acids (VFA) by 45%–52%. Therefore, bioaugmentation is an advantageous approach to enhance biogas production, as it can directly increase the abundance of specific methanogen or acetate-oxidizing bacteria. This approach is increasingly receiving attention in research.

VFAs accumulation, particularly acetate and propionate acid, is commonly considered a major inhibitory factor in AD systems[18]. Propionate acid is a crucial organic VFA because its consumption depends on hydrogen partial pressure and acetate levels[19]. Previous studies have acclimated methanogenic cultures for propionate acid degradation[20,21] to boost AD treating FW at high OLR[22,23] by accelerating VFAs degradation. However, there are few studies on bioaugmentation with methanogenic cultures to start anaerobic digestion of food waste at high OLR.

In this study, OLR of anaerobic digester was gradually increased to determine the start-up threshold. Four semi-continuous AD reactors (i.e., a control group without bioaugmentation and experimental groups with different bioaugmentation strategies) were conducted at the selected start-up OLR to evaluate the bioaugmentation performance. Additionally, 16S rRNA gene sequencing was performed to reveal the changes in the microbial composition before and after bioaugmentation. This study aims to provide insight into whether bioaugmentation strategies can shorten the start-up phase and boost performance for AD of food waste.

## 2 Materials and methods

### 2.1 Feedstock, inoculum, and bioaugmentation seed

The FW used in this study was obtained from the canteen at Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, China. The FW primarily consisted of table waste and underwent a preparation process to remove bones, plastic, napkins, and other non-food waste. The FW was stored at –20°C until use. The FW had the following composition: carbohydrate content of 53.60%±0.20%, lipid content of 12.56%±0.48%, and protein content of 26.38%±0.79%. The inoculum used in the experiment was collected from the food waste treatment biogas plant (Foshan Hanlan Green Electric, China). The biogas plant operated at 37°C, and the inoculum had previously been used to initiate a batch mesophilic reactor, demonstrating its good capability for methane production[24]. Prior to use, the inoculum was degassed.

The bioaugmentation seed (BS) were collected from a 75 L continuous stirred tank reactor (CSTR) (Kemi, China) fed with a basic nutrient medium containing propionic acid at an organic loading rate of 0.5 g VS/L·d, located at Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences. The nutrient medium contained the following ingredients (mg/L): $NH_4Cl$ (400); $MgSO_4·6H_2O$ (250); KCl (400); $CaCl_2·2H_2O$ (120); $(NH_4)_2HPO_4$ (80); $FeCl_3·6H_2O$ (55); and the trace element salts (i.e., $CoCl_2·6H_2O$, $NiCl_2·6H_2O$, $MnCl_2·4H_2O$, $CuCl_2·2H_2O$, $AlCl_3·6H_2O$, $ZnCl_2$, $Na_2WO_4·2H_2O$, $H_3BO_3$, $Na_2SeO_3$, and $Ma_2MoO_4·2H_2O$) (each at 0.5). The BS reactor had stably run for more than 300 d with the methane production of 0.15 L/L·d. The predominant bacteria were *Methanothrix* and *Syntrophobacter*, as reported in the recent study[20,25]. The basic characteristics of FW, inoculum, and BS are listed in Table 1.

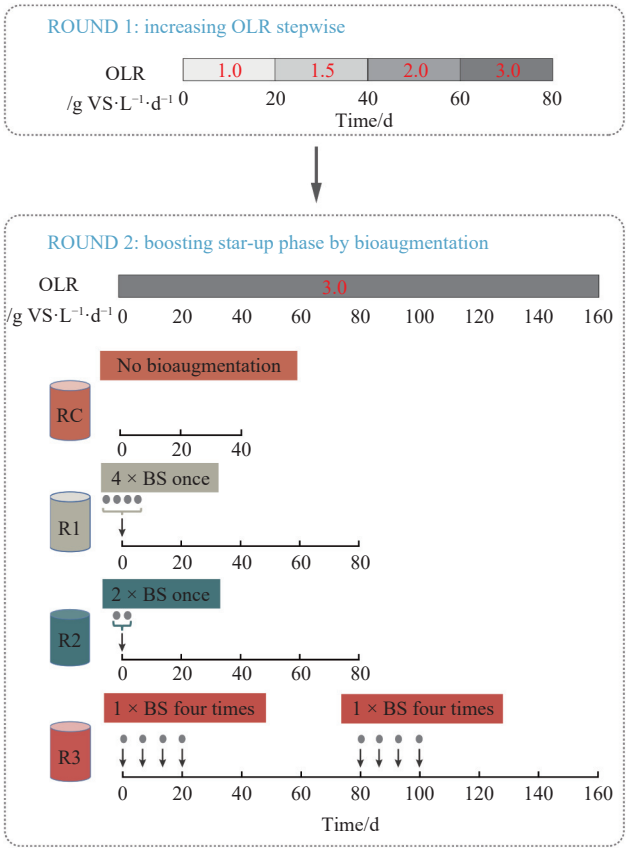
### 2.2 Experimental set-up and procedures

Two rounds of experiments were conducted as shown in Figure1. In the first round, a group of 2 L mesophilic CSTR reactors with a working volume of 1.8 L were operated for 80 d (an HRT of 20 d) to determine the threshold of the OLR-stressed AD process. The initial OLR was set at 1.0 g VS/L·d, then was increased to 1.5 g VS/L·d at the second HRT, 2.0 g VS/L·d at the third HRT, and 3.0 g VS/L·d at the fourth HRT, respectively. All reactors were flushed with $N_2$ to remove headspace air at the beginning. The biogas yield was measured using a 100 mL syringe, and biogas content was recorded every 3 d. Liquid samples were collected every 8 d for pH and VFAs analysis.

**Table 1 Basic characteristics of raw materials, inoculum, and bioaugmentation seed**

| Parameters | Food waste | Inoculum | Bioaugmentation seed |
|---|---|---|---|
| pH | - | 7.65±0.05 | 7.80±0.04 |
| TS/% | 22.35±0.32 | 1.91±0.21 | 1.35±0.09 |
| VS/% | 21.01±0.11 | 1.04±0.25 | 0.41±0.04 |
| C/% | 53.02±0.02 | - | - |
| N/% | 2.57±0.09 | - | - |
| H/% | 7.90±0.07 | - | - |
| C/N | 20.86±0.92 | - | - |

Note: Data are presented as mean±standard deviation ($n$=3); TS: total solids; VS: volatile solids; "-" not detected.




Note: 1×=0.675 g VS/L

Figure 1 Main operational conditions of experimental reactors

In the second round of experiments, four groups (R1, R2, R3, and RC) were established to compare the effectiveness of different bioaugmentation strategies in boosting the start-up phase. Mesophilic CSTRs with a working volume of 1.8 L were started up using the same inoculum as in the first round. The reactors were fed once a day with an OLR of 3.0 g VS/L·d and an HRT of 20 d. The whole process lasted for 160 d and was divided into four phases: Phase I (0-20 d), Phase II (20-80 d), Phase III (80-100 d), and Phase IV (100-160 d). During phase I, bioaugmentation was performed on

R1, R2, and R3. Specifically, bioaugmentation involved centrifuging the BS at 4000 r/min for 5 min, followed by decanting the resulting supernatant to collect the microbial biomass precipitate for use in bioaugmentation. Bioaugmentation dosages were reported between 4%-35% for AD of chicken manure and straw, and the dosage of 0.675 g VS/L was set based on the pre-experiment results of batch AD tests[24-26]. Specifically, in R1, BS at a concentration of 2.700 g VS/L was added once at the beginning of the first HRT. In R2, BS at a concentration of 1.350 g VS/L was added once at the beginning of the first HRT. In R3, BS at a concentration of 0.675 g VS/L was added four times every 5 d (total 2.700 g VS/L) during the first HRT, and the same bioaugmentation process was repeated during the fifth HRT. The reactor without the addition of BS was set as the control group (RC). After the first HRT with bioaugmentation, the reactors ran for the subsequent three HRTs to evaluate the efficiency of bioaugmentation (Figure 1).

### 2.3 Analytical methods

TS and VS were determined using standard methods[27]. C, N, and H contents were measured using a Vario EL element for analysis (Elementar Analysensysteme GmbH, Hanau, Germany). The pH was measured using a METTKER TOLEDO (FE28) portable meter (Mettler Toledo, Swiss) with a glass electrode calibrated in buffers at pH 4.01, 7.00, and 9.21. Alkalinity was detected with 0.25 mol/L $H_2SO_4$ to endpoints at pH 5.7 and 4.3, allowing total, partial, and intermediate alkalinity to be calculated. The VFAs concentration was measured using a high-performance liquid chromatography system (e2698, Waters, USA )[28]. The FW composition (carbohydrate, lipid, and protein) was measured according to GB-5009.5, GB-5009.6 and GB-5009.8. A Bio-RAD column is equipped in this system at a temperature of 50°C. The mobile phase was 0.5 mmol $H_2SO_4$ at a flow rate of 0.5 mL/min. Biogas production was measured using a 100 mL syringe every 2 d, and 100 mL of biogas was extracted to analyze its methane content by gas chromatography (GC-2014, Shimadzu, Kyoto, Japan) equipped with a thermal conductivity detector at a temperature of 120°C and a Porapak Q column at a temperature of 70°C, and the carrier gas was argon (20 mL/min)[25]. Statistical analysis was calculated by Microsoft Excel 2016.

### 2.4 16S rRNA gene amplification

Samples from R1 (days 0, 30, 40, 60, and 80), R2 (day 0, 30, 40, 60, and 68), R3 (days 0, 30, 40, 60, 80, 100, 120, 140, and 160), and RC (days 0 and 30) were collected for microbial dynamic shift analysis. As previously described[29], DNA extraction and 16S rRNA gene sequencing were carried out. The resulting sequences were systematically assigned to phyla, classes, and genera. The results were included to compare and analyze the microbial community structure between the bioaugmentation and control groups. Sequence data were deposited in the National Center for Biotechnology Information (NCBI) Short Read Archive database (accession number: PRJNA849406).

## 3 Results and discussion

### 3.1 Start-up performance of anaerobic digestion of food waste by increasing the organic loading rate stepwise

To investigate the effect of OLR on AD during the start-up phase, a gradual increase in the OLR was applied. Figure 2 shows that AD system failed at an OLR of 3.0 g VS/L·d, as evidenced by a significant decline in volumetric biogas production (VBP) and methane yield. This failure can be attributed to the accumulation of high levels of VFAs, specifically acetate (1716.50±139.50 mg/L) and propionate (2204.50±157.50 mg/L), at the OLR of 3.0 g VS/L·d. This observation aligns with a previous study that reported AD collapses at acetate levels of 1600 mg/L and propionate levels of 900 mg/L[30]. Propionate concentrations exceeding 900 mg/L can lead to severe damage to the AD system, primarily due to the difficulty of its degradation, particularly under low pH conditions[31]. Thus, an OLR of 3.0 g VS/L·d exerted an inhibitory effect on AD, promoting subsequent bioaugmentation experiments aimed at boosting start-up phase.

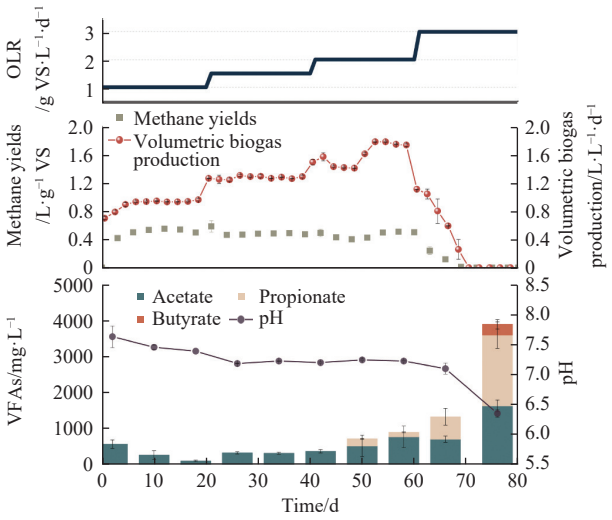



Figure 2 The start-up performance of food waste AD by increasing OLR stepwise

### 3.2 Effects of bioaugmentation during high OLR start-up

The methane yield of the RC exhibited a sharp decrease and eventually dropped to zero on day 27, indicating a system collapse. By contrast, the methane yield of all bioaugmented reactors was higher than that of RC (Figure 3). Besides, the average methane yield of R1 (4× BS, once every four HRTs, 0.48 L/g VS), R2 (2× BS, once every four HRTs, 0.41 L/g VS), and R3 (1× BS, 4 times every four HRTs, 0.53 L/g VS) were 0.26, 0.05, and 0.39 times higher, respectively, than that of the reactor stepwise increased OLR to 3.0 g VS/L·d in the first round (0.38 L/g VS) during the first four HRTs. Thus, these bioaugmentation strategies were effective for quickening and boosting start-up of the high-OLR system, while a long time was needed for gradual stabilization of general reactors in previous reports[2,6]. In addition, among the three bioaugmented digesters, R3 with high-frequency bioaugmentation not only exhibited the best performance in increasing methane yield, but also maintained a steady phase lasting for four HRTs, indicating that multiple-frequency bioaugmentation was more effective than one-time bioaugmentation. Therefore, the frequency and dosage of the BS addition play a crucial role in maintaining the stability of AD start-up at high OLR by increasing the immobilization of propionate-utilizing cultures. Considering the slight decline in methane yield of R3 during the fourth HRT, the same bioaugmentation strategy was implemented during the fifth HRT. The methane yield from day 80 to 160 followed a similar trend to that from day 0 to 80. Hence, bioaugmentation every four HRTs is an effective strategy to maintain a high methane yield.

The alkalinity ratio, defined as the ratio between intermediate and partial alkalinity (IA:PA), is a useful indicator of the stability of the AD system. An IA:PA value exceeding 0.9 suggests a high risk of system failure[32]. As shown in Figure 4, the value of IA:PA in RC increased sharply at the beginning and reached to 1.28±0.20 on day 28, indicating system instability. The IA:PA values of R1 and R2

ranged from 0.40 to 0.60 during day 0-20 bioaugmentation phase and the following 20 d, after which they exceeded 0.90, suggesting a loss of efficacy in the bioaugmentation strategies, consistent with the aforementioned biogas production. In contrast, the IA:PA of R3 remained within the range of 0.51 to 0.59 throughout the entire experimental period, suggesting a stable system.

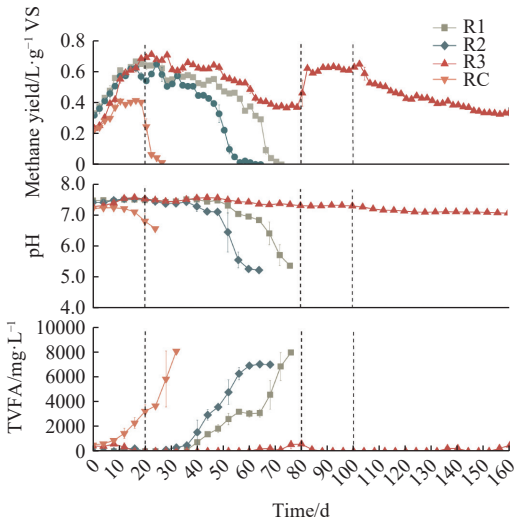



Figure 3 Bioaugmentation performance of the semi-continuous anaerobic digestion process

During the first HRT, there was no significant accumulation of VFAs in the bioaugmented digesters compared to RC. However, after running for two HRTs, the total VFAs accumulated in R1 (6853.50±1130.50 mg/L) and R2 (7036.50±26.50 mg/L), primarily consisting of acetic acid and propionic acid (Figure 5). These observations were consistent with previous studies where propionate accumulation occurred when R1, R2, and RC were on the verge of collapse[33,34]. In contrast, the total VFAs concentration in R3 remained below the defined healthy concentration (<1500 mg Hac/L) throughout the experimental period (Figure 3), indicating system stability. Thus, an optimal dosage and frequency of BS addition effectively prevented total VFAs accumulation in the reactors under high OLR. The change in pH was associated with the change in total VFAs, that is, the accumulation of total VFAs led to a drop in pH. As shown in Figure 3, the pH of RC sharply decreased to 6.57±0.24, which was detrimental to methanogen growth and limited the methanogenesis process, which was caused by accumulation of VFAs and led to decrease in methane production (Figure 3 and 4). The pH of R1 and R2 remained stable during the first two HRTs but decreased in the third HRT, indicating the failure of BS addition. In comparison, the pH of R3 fluctuated between 7.00 and 7.50 throughout the entire experimental period, indicating that BS addition with optimal dosage and frequency contributed to a good buffer capacity in the AD system.

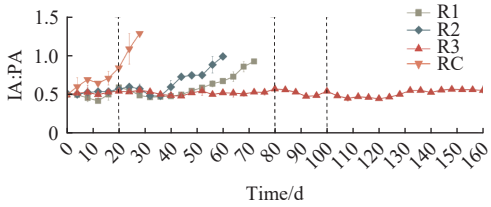



Figure 4 The alkalinity ratio of the semi-continuous anaerobic digestion process

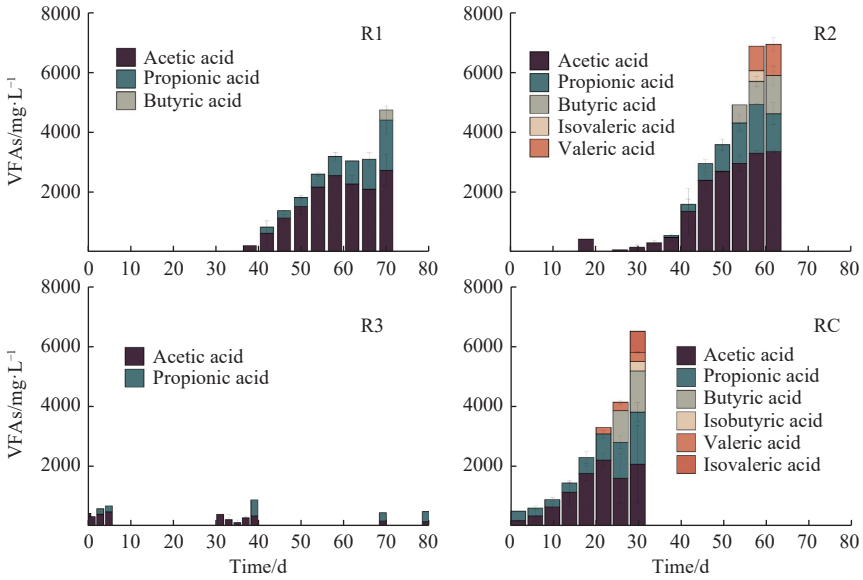


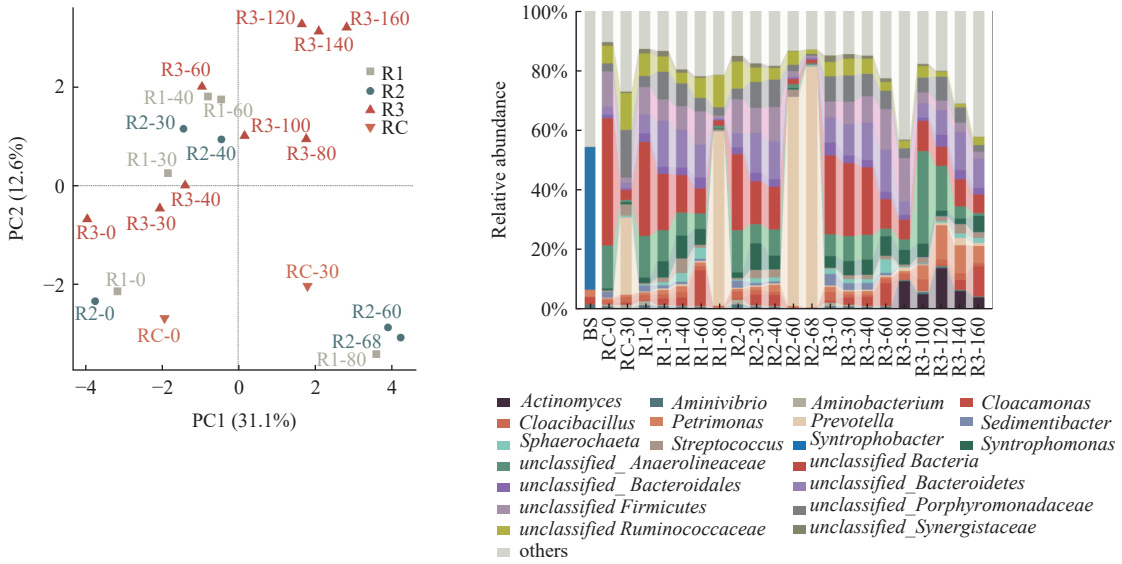

Figure 5 The concentration of VFAs in R1, R2, R3, and RC

Overall, bioaugmentation proved to be effective in enhancing the start-up phase of anaerobic digestion of food waste under high OLR conditions, resulting in improved biogas production, VFAs degradation, and buffer capacity. Furthermore, the success of bioaugmentation was found to be closely related to the frequency and dosage of addition. The bioaugmentation strategy with high frequency and high dosage, as demonstrated by R3 (1× BS 4 times every four HRTs), not only exhibited the best performance but also sustained its effect over a longer period.

### 3.3 Influence of bioaugmentation on bacterial community composition

Beta diversity analysis revealed that the bacterial composition of R3 differed from that of the other two bioaugmented reactors (Figure 6a), indicating that the bioaugmentation effect varied depending on dosage and frequency. In addition, there was a difference between the initial and final bacterial composition of R3. This suggests that the BS was well-suited to the inoculum consortia and gradually adapted to the high-OLR system. In contrast, the BS in R1 and R2 were incompatible after two HRTs.

The relative abundance of the bacterial communities at the




a. Beta diversity change b. Microbial community abundance on genus level

Note: Principal components (PC) 1 and 2 represented 31.1% and 21.6% of community variation, respectively.

Figure 6 The effect of bioaugmentation on bacterial community

genus level across different groups is shown in Figure 6b, providing insight into the influence of bioaugmentation. At the beginning stage, no remarkable differences were found in the major bacteria among all reactors. However, different bioaugmentation strategies had similar but varying effects on microbial populations. Specifically, the increased and dominant genera included *Syntrophomonas* (0.15%-8.89%), *Candidatus Cloacamonas* (0.05%-12.09%), *Petrimonas* (0.08%-11.41%), *Prevotella* (0.22%-80.82%), and some unclassified bacteria. *Streptococcus*, which can convert carbohydrates to glucose, showed an increasing trend in relative abundance (approximately 4.5%), contributing to the efficient degradation of FW[35,36]. *Prevotella* was predominant in all reactors as an efficient player in degrading proteins[37]. VFAs started to accumulate notably as the abundance of *Prevotella* increased, which was consistent with earlier findings[38]. The relative abundance of *Syntrophomonas* increased by 0.88%-3.60% in all bioaugmented reactors on day 30, which enhanced the syntrophic conversion rate of butyrate to acetate[39]. Similarly, *Candidatus Cloacamonas* was also enriched in the bioaugmented digesters, which participate in the oxidation of propionate to acetic acid, $CO_2$, and $H_2$[40,41]. The establishment of these genera indicated that the introduction of bioaugmentation was prominent in propionate and butyrate degradation. Although the Shannon and Simpson diversity indices were similar among all bioaugmented groups, there is still a need to highlight that a higher frequency of BS addition to R3 led to a more stable microbial community during Phase IV (100-160 d) compared to the other reactors ($p$<0.01, Table 2).

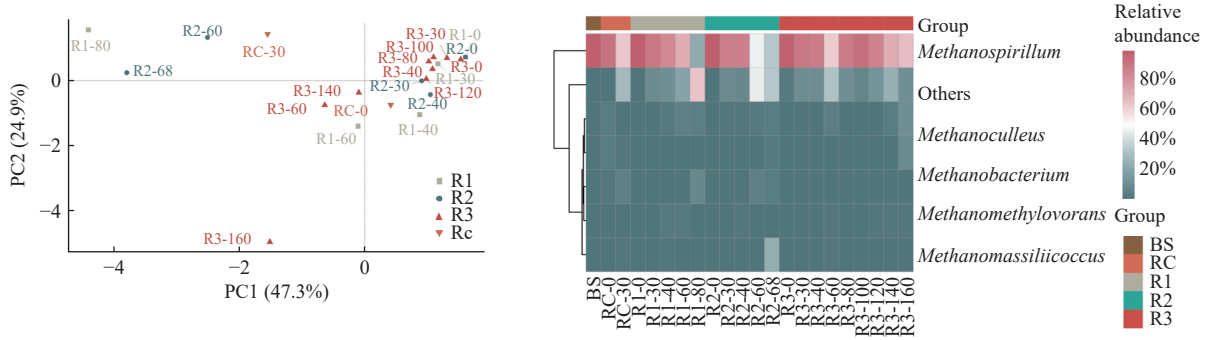
### 3.4 Influence of bioaugmentation on methanogen community composition

As shown in Figure 7a, the methanogenic communities in the early stage of reactors were similar, but differences appeared between the well-performing R3 and the three above reactors. To gain a better understanding of the effects of different bioaugmentation strategies, Figure 7b shows methanogenic populations in the four digesters at the genus level. *Methanothrix*, a strict aceticlastic methanogen, was the most abundant methanogenic player across all bioaugmented reactors when VFAs were below 6000 mg/L, ranging from 70% to 99% at the genus level. Its dominant role contributed to the efficient conversion of acetate to methane and subsequently promoted propionate degradation

**Table 2 Index for Shannon, Chao, Ace, and Simpson from semi-continuous digesters**

| Reactor | Day | Shannon | Chao | Ace | Simpson |
|---|---|---|---|---|---|
| R1 | 0 | 4.052 | 971.415 | 990.384 | 0.054 |
| | 30 | 4.383 | 932.082 | 942.501 | 0.030 |
| | 40 | 4.456 | 971.515 | 993.826 | 0.026 |
| | 60 | 4.302 | 935.189 | 975.288 | 0.033 |
| | 80 | 2.882 | 798.100 | 812.214 | 0.184 |
| R2 | 0 | 4.321 | 856.958 | 846.852 | 0.034 |
| | 30 | 4.472 | 993.722 | 1011.550 | 0.026 |
| | 40 | 4.339 | 936.102 | 948.233 | 0.028 |
| | 60 | 1.996 | 794.039 | 757.055 | 0.458 |
| | 68 | 1.624 | 718.414 | 712.373 | 0.555 |
| R3 | 0 | 4.223 | 916.525 | 939.775 | 0.049 |
| | 30 | 4.463 | 967.842 | 971.514 | 0.028 |
| | 40 | 4.494 | 952.450 | 967.636 | 0.025 |
| | 60 | 4.380 | 955.783 | 954.627 | 0.028 |
| | 80 | 3.580 | 937.858 | 942.925 | 0.097 |
| | 100 | 4.030 | 932.552 | 942.078 | 0.056 |
| | 120 | 4.046 | 832.960 | 832.517 | 0.048 |
| | 140 | 4.332 | 902.206 | 901.162 | 0.032 |
| | 160 | 4.159 | 825.308 | 839.410 | 0.039 |
| RC | 0 | 3.586 | 866.805 | 883.771 | 0.096 |
| | 30 | 3.550 | 859.103 | 855.404 | 0.068 |

without any VFA accumulation (Figure 5), which is consistent with a previous study[42]. By comparing the three bioaugmented reactors, it was found that the duration of stable methane production was determined by the abundance of introduced *Methanothrix*. For example, the longest stable performance, up to 160 d, was observed in R3 (1× BS 4 times every four HRTs), which received the highest amount of *Methanothrix* addition, followed by 72 d in R1 (4× BS once every four HRTs), and 64 d in R2 (2× BS once every four HRTs). These results suggest that the amount of *Methanothrix* introduced to R1 and R2 was insufficient to avoid the accumulation of acetate when the OLR was 3.0 g VS/L·d. The dominant role of *Methanothrix* in R1 and R2 was gradually replaced by the hydrogenotrophic methanogen *Methanospirillum* once total VFAs exceeded 6000 mg/L. Therefore, 6000 mg/L was concluded to be the inhibitory threshold level for *Methanothrix*.




a. Beta diversity change b. Heatmap of microbial community abundance on genus level

Note: Principal components (PC) 1 and 2 represented 47.3% and 24.9% of community variation, respectively.

Figure 7 The effect of bioaugmentation on Archaeal community

*Methanospirillum* was the second most dominant methanogen when the total VFAs concentration was less than 6000 mg/L in all reactors. Its relative abundance continued to increase and even became the most dominant methanogen when total VFAs concentrations were more than 6000 mg/L in R1, R2, and RC. Surprisingly, its dominant role was accompanied by a decline in methane production and an increase in total VFAs, especially in acetate and propionate, from day 60 in R1, day 60 in R2, and day 30 in RC. This indicated that in the VFA-accumulated reactors, hydrogenotrophic methanogens played a more important role in assisting propionate-oxidizing syntropy to overcome thermodynamic barriers and degrade propionate by scavenging $H_2$ rather than generating methane by oxidizing acetate. The trace proportion of $H_2$ in the anaerobic system led to slow methanogenesis.

Thus, it is necessary to highlight that acetogenesis and hydrogenotrophic pathways should coexist in high-load anaerobic reactors. The key to successful bioaugmentation is to regularly provide mixed functional consortia, i.e., a large proportion of acetogenic methanogens and a trace amount of hydrogenotrophic methanogens.

## 4 Conclusion

This study has demonstrated that the introduction of methanogenic cultures succeeds in starting and maintaining semi-continuous AD with high-OLR food waste. The results have shown that bioaugmentation with a high dose of methanogens is essential for achieving efficient start-up and stable operation of the anaerobic digester for at least four HRTs. In addition, the optimal bioaugmentation strategy should include a large proportion of acetoclastic methanogens, *Methanothrix*, and a small proportion of hydrogenotrophic methanogens, *Methanospirillum*, to maintain VFAs under inhibitory levels and achieve high methane yield. These findings provide a valuable starting point for the application of bioaugmentation to achieve a rapid and stable start-up of anaerobic digestion at high organic loading rates. However, the feasibility of using bioaugmentation in industrial-scale reactors depends on the input-to-output cost ratio. Therefore, further research is needed to investigate low-cost bioaugmentation methods and conduct an economic evaluation in the near future.

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