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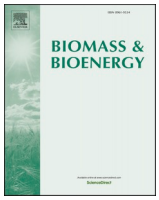

Enhanced methane production from food waste: A systematic comparison between conventional single-stage and lactate-based two-stage anaerobic digestion processes

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ABSTRACT

The increasing generation of food waste (FW) poses significant environmental and management challenges, requiring efficient and sustainable treatment methods. This study presents the first systematic comparison between a conventional single-stage anaerobic digestion (AD) process and a lactate-based two-stage AD process using food waste (FW) as the substrate. Both AD configurations were operated in parallel under identical operating conditions, i.e., 37 °C, 20 days hydraulic retention time, 2.3 g volatile solids (VS)/L-d organic loading rate, and pH 8. The two-stage AD system exhibited a methane productivity of 959 NmL CH_4/L-d and a methane yield of 398 NmL CH_4/g VS_{fed}, which were 32.0 ± 5.6 % and 35.9 ± 0.6 % higher than those of the single-stage AD process, respectively. The two-stage AD system also showed significant lactate accumulation in the acidogenic stage, which was almost completely oxidized in the methanogenic stage. Furthermore, molecular analysis of the acidogenic stage revealed diverse bacterial communities, with a prevalence of lactate-producing bacteria such as *Lactobacillus*. In the methanogenic stage, various bacteria and archaea, including *Methanobacterium* and *Methanothrix*, were identified as major contributors to methane production. The enhanced methane production performance of the two-stage AD system was attributed to the physical separation of the acidogenic stage from methanogenesis and the occurrence of lactate-type fermentation in the acidogenic stage.

1. Introduction

Food waste (FW) management is a critical global challenge, with 931 million tons generated worldwide in 2019, mostly ending up in landfills or incinerators. This represents a cost of 143 billion euros and significant carbon and energy losses [1,2]. Anaerobic digestion (AD) is recognized as a cost-effective and eco-friendly technology for recovering carbon, nutrients, and producing energy from FW [3,4]. However, to ensure its competitiveness in a circular bioeconomy, novel strategies to improve AD efficiency are needed. Traditional single-stage AD systems, although widely used due to their simplicity, may not fully exploit the potential of FW due to differences in microbial communities between the hydrolytic/acidogenic and methanogenic stages. Two-stage AD systems offer improved efficiency, with recent studies suggesting lactate-type fermentation as a favorable approach due to its thermodynamic advantages [5,6]. In this context, the Gibbs free energy obtained, at standard physiological conditions, after the anaerobic oxidation of lactate to acetate and hydrogen (−8.4 kJ/mol) exhibits superior thermodynamics in comparison to propionate (+152 kJ/mol), butyrate (+48 kJ/mol), or ethanol (+19 kJ/mol) [6,7]. Lactate-based AD has emerged as a promising configuration, improving methane (CH_4) yield by 20–58 % and CH_4 content by 11–19 % [8]. Recently, García-Depraect et al. [9] conducted a study on a lactate-based two-stage AD batch process using FW as a feedstock. The results showed that the two-stage AD configuration yielded approximately 425.3 NmL/g volatile solids (VS) fed, a 13 % improvement over the single-stage counterpart under optimal conditions. Despite the potential benefits, there is limited research on lactate-based two-stage AD processes. This study represents the initial effort to systematically compare a conventional one-stage AD process with a two-stage lactate-based process.

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2. Material and methods

2.1. Substrate and inocula

The substrate was formulated to replicate restaurant FW, as previously described by Regueira-Marcos et al. [10], consisting of potato (78 %), chicken breast (14 %), white cabbage (4 %), and pork lard (4 % w/w). These ingredients were blended and the resulting mixture was stored at −20 °C until use. The pH of the FW was 6.3 ± 0.1, and the chemical oxygen demand (COD) and total solids (TS) concentrations were 295 g COD/kg and 211 g TS/kg, respectively. In the hydrolysis-acidogenesis stage, a mixed culture obtained from a pilot-scale anaerobic digester operated with FW under mesophilic conditions was used as inoculum after a heat shock pretreatment at 80 °C for 20 min. This inoculum was stored at 4 °C and reactivated as previously described by Martínez-Mendoza et al. [11], resulting in a concentration of 180 mg volatile suspended solids (VSS)/L. In addition, fresh mesophilic anaerobic sludge, collected from the municipal wastewater treatment plant of Valladolid, Spain, was used as the methanogenic inoculum. The methanogenic inoculum was preincubated at 37 °C for 7 days prior to inoculation. The methanogenic inoculum had a pH of 7.5 and TS and VS contents of 29.7 g/L and 14.9 g/L, respectively.

2.2. Experimental set-up and process operation

In this study, a one-stage AD system was evaluated using a 6.25 L custom-made reactor with a working volume of 5.0 L. Simultaneously, a two-stage system was operated, where the hydrolysis-acidogenesis stage was evaluated in a 1.25 L custom-made fermenter (1 L working volume). Subsequently, the fermentation broth was then transferred to a 5 L reactor with a working volume of 4.0 L. Both AD configurations were operated continuously for 40 days under the same conditions with a hydraulic retention time (HRT) of 20 days (in the two-stage system, the HRT was set to 4 days in the acidogenic stage and to 16 days in the methanogenic stage) at 37 ± 1 °C, 200 rpm, and without pH control (maintained at 3.5 ± 0.1 in the acidogenic reactor and at 8.0 ± 0.2 in both methanogenic reactors). The substrate feed concentration was kept constant at 47.5 g VS/L, equivalent to 5 % TS, while the total organic loading rate (OLR) was 2.3 g VS/L-d. Liquid samples were periodically collected from the effluents of both systems to monitor VS removal, alkalinity, pH, organic acid profile, ammonium ($N–NH_4^+$) and total and soluble chemical oxygen demand (TCOD and SCOD) concentrations. The flow rate and composition of the methanogenic off-gas generated during the AD process were measured daily and reported at standard conditions (0 °C and 1 atm). Key performance indicators included CH_4 concentration in biogas, stability index, and CH_4 productivity and yield. Five samples were collected for analysis of the microbial community composition. For the acidogenic community, E1 from the inoculum and E3 from the final operating time of the first acidogenic reactor were analyzed. For the methanogenic stage, bacterial and archaeal communities were examined in E2 (methanogenic inoculum), and in E5 and E4 from the final operation time of the one-stage and two-stage methanogenic reactors, respectively.

2.3. Analytical methods

Alkalinity, pH, solids, $N–NH_4^+$ and COD were determined using standard methods [12]. Organic acids profile and solids removal were evaluated according to Martínez-Mendoza et al. [13]. Stability index, CH_4 productivity, and yield were reported according to the methodology of García-Depraect et al. [6]. The biogas composition and the microbial taxonomic profile for bacterial composition were previously described by García-Depraect et al. [9]. The degree of acidification was calculated according to Martínez-Mendoza et al. [13]. In addition, the hypervariable region V4 of the 16S rRNA gene was analyzed according to the protocol of Pausan et al. [14], to determine the diversity of archaea in the methanogenic reactors.

3. Results and discussion

3.1. Biogas production and operational performance indicators

The one-stage and two-stage AD systems exhibited an average biogas productivity of up to 1304.8 ± 190.5 NmL/L-d and 1695.0 ± 275.6 NmL/L-d, respectively. Similarly, the CH_4 productivity reached levels of up to 726.6 ± 71.1 NmL CH_4/L-d and 959.3 ± 75.3 NmL CH_4/L-d, respectively. Additionally, CH_4 yields of up to 293.0 ± 35.4 NmL CH_4/g VS_{fed} were recorded for the single-stage configuration, while yields of 398.1 ± 35.2 NmL CH_4/g VS_{fed} were observed for the two-stage configuration (Fig. 1). These yields are consistent with those observed in previous studies on the AD of FW. For example, Kinnunen et al. [15] reported CH_4 yields of 379.7 ± 75.3 NmL CH_4/g VS_{fed} under comparable operating parameters for single-stage AD. Similarly, Liu et al. [16] achieved a CH_4 yield of 371 NmL CH_4/g VS_{fed} under mesophilic conditions. Wu et al. [17] conducted a study on lactate-based two-stage AD of fruit and vegetable waste over a 55-day period at a temperature of 35 °C. That study reported an average CH_4 yield of 261.4 NmL CH_4/g $COD_{removed}$. No measurable gas was detected in the acidogenic reactor of the two-stage system during the entire period of operation. The stability index (0.93 ± 0.02 and 0.92 ± 0.05), biogas composition (CH_4 55.6 ± 2.0 and 55.2 ± 3.2 % *v/v*; carbon dioxide (CO_2) 42.7 ± 1.9 and 43.2 ± 3.1 % *v/v*), pH (8.4 ± 0.4 and 8.6 ± 0.2), and VS removal (83.9 ± 3.4 % and 81.9 ± 7.4 %) showed differences of less than 3.0 % between the single-stage and two-stage configurations. Additionally, similar $N–NH_4^+$ concentrations were observed between the one-stage and two-stage AD systems, with concentrations of 1.1 ± 0.2 and 1.1 ± 0.3 g $N–NH_4$/L, respectively. The two-stage AD system for FW offers significant environmental benefits beyond enhanced CH_4 production. It reduces greenhouse gas emissions by capturing CH_4 that would otherwise escape from landfills. Additionally, the nutrient-rich digestate produced serves as a valuable fertilizer, promoting nutrient recycling and improving soil health. Thus, the two-stage AD process not only increases the efficiency of biogas production but also supports sustainable waste management practices and contributes to a more circular economy.

It is important to mention that the present study was a first attempt to systematically compare the two AD configurations for the methanization of FW. It is necessary to carry out this comparison with a longer operating time and evaluation of different OLRs to validate the advantage of the two-stage configuration over its single-stage counterpart. Also, an economic analysis of both single-stage and two-stage AD systems should be included to better understand the economic impact of implementing a two-stage AD system compared to a single-stage AD process.

3.2. Organic acid profile

In both the one- and two-stage AD systems, lactate, formate, acetate, and propionate were identified as the predominant soluble metabolites, whereas ethanol was not detected (Fig. 2). The average degree of acidification of FW was measured to be 2.2 ± 0.7 %, indicating a low pre-acidification. Throughout the methanogenic stage, the organic acid profile remained similar in both systems, with acetate and propionate gradually increasing up to 4.0 and 1.8 g/L, respectively, in the one-stage system, and up to 1.6 g/L for both acids in the two-stage system. Notably, a significant presence of lactate was consistently observed in the acidogenic reactor throughout the experimental period, reaching an average concentration of 6.5 ± 0.9 g/L, which was degraded by 99.9 ± 0.03 % during the methanogenic stage. According to the thermodynamics of AD pathways involved in the conversion of hexose to CH_4, acetogenic bacteria and methanogenic archaea can yield more energy when lactate serves as the acidogenic end product. Primary fermentation of lactate results in a Gibbs free energy change ($\Delta G^{0\prime}$) value of −65.8 kJ per mole of CH_4 produced under standard physiological

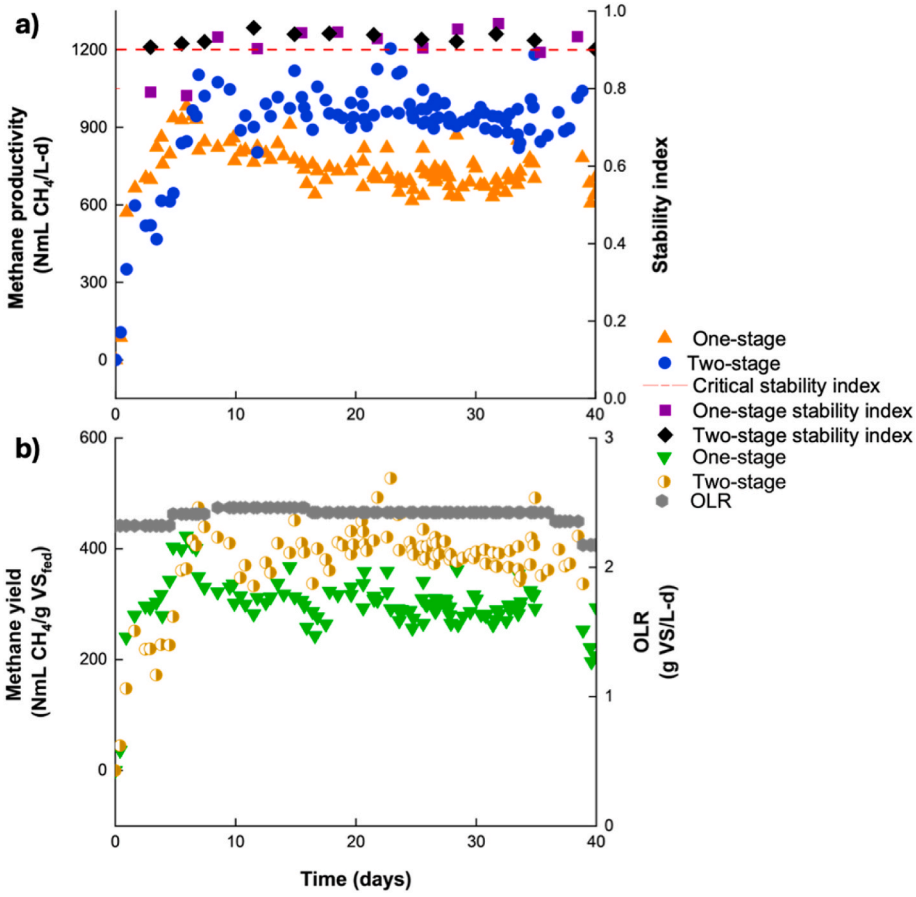

Fig. 1. Time course of a) CH_4 productivity and stability index and b) CH_4 yield and organic loading rate (OLR), recorded during the systematic comparison between one-stage and lactate-based two-stage AD of FW.

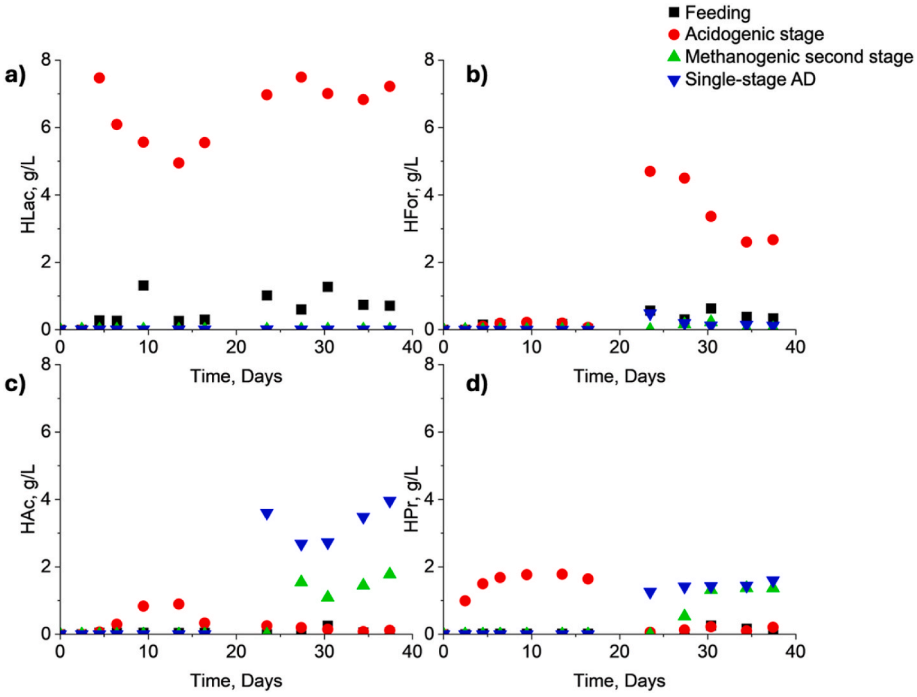

Fig. 2. Time course of a) lactic acid (HLac), b) formic acid (HFor), c) acetic acid (HAc), and d) propionic acid (HPr), recorded during the systematic comparison between one-stage and lactate-based two-stage AD of FW.

conditions, which allocates more energy to methanogens compared to butyric-type fermentation (−32.6 kJ), acetic-type fermentation (−31 kJ), propionic-type fermentation (−32.1 kJ), and ethanol-type fermentation (−59.4 kJ) [7,9].

3.3. Microbial ecology

In the acidogenic reactor, the microbial diversity in E1 was dominated by *Bacteroides*, *Clostridium*, *Enterobacter*, *Leuconostoc*, *Klebsiella* and *Pseudomonas* with a relative abundance of 50.7, 19.1, 14.2, 8.9, 3.6 and 3.3 %, respectively, accounting for >99 % of the total community (Fig. 3). In sample E3, there was a notable variation in the bacterial community, comprising *Lactobacillus*, *Limosilactobacillus*, *Weisella*, *Lactococcus*, *Leuconostoc*, and *Serratia* with relative abundances of 58.5, 30.5, 4.1, 1.9, 1.6, and 1.0 %, respectively, which together accounted for over 97 % of the total community. Thus, lactate production was clearly supported by the predominant activity of lactic acid bacteria.

The methanogenic microbial diversity of bacteria and archaea was analyzed in three samples. In E2, the bacterial community was composed of *Mycolicibacterium* (5 %), *Leptolinea* (3.5 %), *Romboutsia* (3.2 %), *Pirellulaceae Pir4 lineage* (3 %), *Clostridium* (2.5 %), *Longilinea* (2.3 %), *Thermovirga* (1.5 %), *Candidatus Caldatribacterium* (1.4 %), *Williamwhitmaniaceae Bacteroidales* (1.3 %), *Microthrixaceae* IMCC26207 (1.2 %), *Phycicoccus* (1.1 %), *Pedosphaeraceae ADurb Bin063 1* (1.1 %), *Candidatus Cloacimonas* (1 %), and *Rikenellaceae DMER64* (1 %), together representing 29 % of the total community. The archaeal population in E2 was represented by *Candidatus Methanofastidiosum*, *Methanomassiliicoccus*, *Methanobrevibacter*, *Methanothrix*, *Methanospirillum*, *Methanobacterium*, *Candidatus Methanomethylicus*, *Methanosphaera*, *Methanolinea*, *Candidatus Nitrocosmicus*, *Methanosarcina*, and *Methanomethylovorans,* with relative abundances of 17.0, 15.2, 14.2, 13.9, 9.7, 7.6, 4.2, 3.6, 3.4, 1.2, 1.1, and 1.0 %, respectively, together accounting for 92.1 % of the total community.

In E4, the bacterial diversity was represented by *Paludicola*, *Comamonas*, *Paenalcaligenes*, *Cloacimonadaceae W5*, *Oblitimonas*, *Thiopseudomonas*, *Thermovirga*, *Bellilinea*, and *Syntrophomonas,* with relative abundances of 7.8, 5.0, 4.4, 3.9, 3.2, 3.0, 1.8, 1.6, and 1.4 %, respectively, together accounting for 32 % of the total community. On the other hand, the archaeal microbial community in E4 was composed of *Methanobacterium*, *Methanothrix*, *Methanomassiliicoccus*, *Candidatus Methanofastidiosum*, *Methanospirillum*, *Methanoculleus*, and *Methanomethylovorans,* with relative abundances of 22.7, 20.6, 15.8, 15.5, 8.5, 6.5, and 3.2 %, respectively, together accounting for 92.8 % of the total community.

The final sample in the one-stage AD system (E5) showed a diverse bacterial microbial ecology, consisting of *Paludibacter*, *Longilinea*, *Proteiniphilum*, *Flexilinea*, *Thermovirga*, *Bellilinea*, *Leuconostoc*, *Paludicola*, and *Leptolinea,* with relative abundances of 4.5, 3.4, 3.3, 2.9, 2.8, 2.3, 1.2, 1.2, and 1.0 %, respectively, which together accounted for 22.5 % of the total community. The archaeal microbial community in E5 included *Methanobacterium*, *Candidatus Methanofastidiosum*, *Methanothrix*, *Methanomassiliicoccus*, *Methanoculleus*, and *Methanospirillum,* with relative abundances of 27.1, 22.0, 14.9, 6.2, 2.1, and 1.1 %, respectively; together these taxa accounted for 73.4 % of the total archaeal

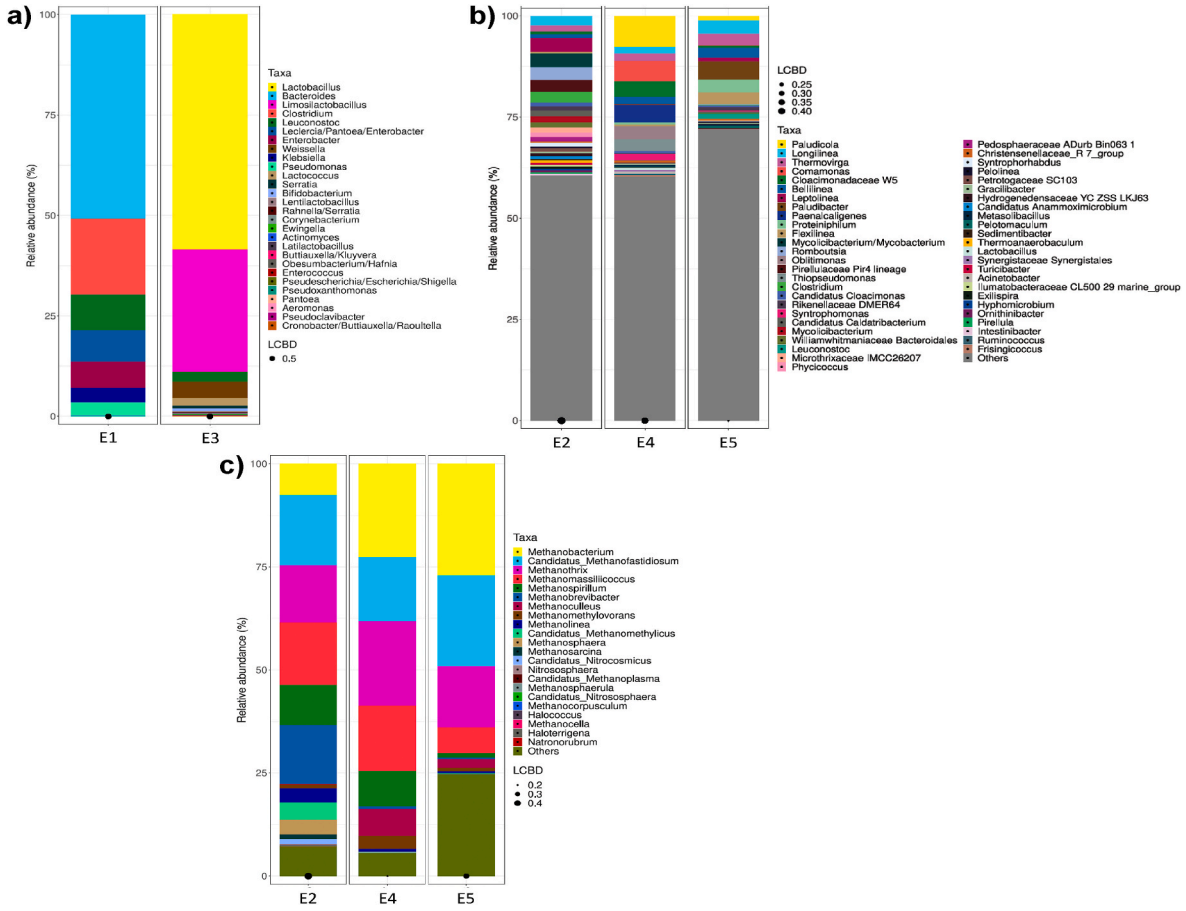

Fig. 3. Microbial community structure at the genus level for a) bacteria in the acidogenic reactor for both the inoculum (E1) and the final sample (E3), b) bacteria in the methanogenic inoculum (E2) and the final sample at the methanogenic stage for both the two-stage system (E4) and the one-stage system (E5), c) archaea in the methanogenic inoculum (E2) and the final sample at the methanogenic stage for both the two-stage system (E4) and the one-stage system (E5). Local contribution to beta diversity (LCBD) represents a dissimilarity coefficient, with higher LCBD values indicating increased dissimilarity among the samples examined.

community. *Methanobacterium* probably played a significant role in CH_4 production from hydrogen and CO_2, along with *Methanospirillum* and *Methanoculleus*, both classified as hydrogenotrophic methanogens. These species may have emerged as dominant contributors due to acetic acid depletion [18]. Additionally, *Methanothrix*, known as an aceto-clastic methanogen, possesses the ability to convert acetate to CH_4 [19]. The predominant bacteria have been identified for their distinct roles in anaerobic environments. *Paenalcaligenes*, as documented by Feng et al. [20], has been recognized as producers of short-chain fatty acids under such conditions. Additionally, *Comamonas* spp., as shown by Camargo et al. [21], are involved in the production of acetic acid. *Longilinea*, classified in the phylum Chloroflexi, has been implicated in the metabolism of various carbohydrates to produce organic acids [22]. Finally, *Proteiniphilum*, a member of the phylum Bacteroidetes, has been shown by Perman et al. [23] to degrade both peptides and complex carbohydrates. Future research should investigate the dynamics of microbial populations, particularly their ability to adapt to real process conditions, which will also be critical in predicting long-term stability. In this sense, the robustness and resilience of the lactate-based two-stage AD of FW should also be tested against typical fluctuations/perturbations in key operating parameters such as temperature, pH and OLR.

4. Conclusions

The comparison between one-stage and two-stage AD systems revealed notable differences in CH_4 productivity and CH_4 yield. The lactate-based two-stage AD system showed significantly higher CH_4 productivities and yields compared to the one-stage system. However, both systems exhibited similar stability indices, biogas composition, pH levels, and VS removal rates. The marked presence of lactate in the acidogenic reactor, coupled with its efficient degradation in the methanogenic stage, highlights the promising potential of lactate as CH_4 precursor. Lactate-producing bacteria were particularly involved in the acidogenic stage. Both methanogenic stages harbored diverse bacteria and archaea, with *Methanobacterium* and *Methanothrix* involved in CH_4 production. These results highlight the potential of lactate-based fermentation for enhanced biogas production in FW AD systems. Further research is needed to thoroughly investigate and optimize lactate-based two-stage AD systems for efficient FW treatment and resource recovery, particularly through extended operational periods.

CRediT authorship contribution statement

Leonardo J. Martínez-Mendoza: Writing – original draft, Investigation, Formal analysis, Data curation. **Raúl Múñoz:** Writing – review & editing, Supervision, Resources, Project administration. **Octavio García-Depraect:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization.

Data availability

Data will be made available on request.

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