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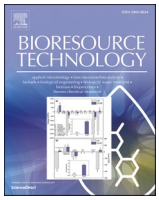

Two-stage anaerobic digestion of food waste: Enhanced bioenergy production rate by steering lactate-type fermentation during hydrolysis-acidogenesis

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HIGHLIGHTS

- Prevailing bacteria and culture pH modulated the acidogenic fermentation pathways.
- Self-fermentation of FW without pH control entailed a prominent build-up of lactate.
- Lactic fermentation resulted in lower volatile solids and acidogenic off-gas losses.
- The lactate-rich acidogenic effluent supported syntrophic biogas production.
- Two-stage AD with primary lactate-type fermentation doubled FW treatment capacity.

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ABSTRACT

This study proposed a lactate-based two-stage anaerobic digestion (AD) process to enhance bioenergy production rate from food waste (FW) and investigated the effect of inoculum addition and culture pH on hydrolysis-acidogenesis and further methanization. A series of batch fermentations were performed with an enriched lactate-producing consortium and without inoculum addition under controlled (5.7) and uncontrolled pH (initial 6.7) conditions. The interplay between the studied factors dictated the fate of lactate, particularly if it is produced and accumulated in the fermentation broth or is consumed by butyrogenic bacteria. Only the self-fermentation of FW with uncontrolled pH resulted in lactate accumulation (0.2 g/g volatile solid (VS) fed) with limited off-gas production (0.32 NL/L) and VS losses (≈16%). Such lactate-rich broth was successfully digested through biochemical methane potential tests, resulting in a maximum bioenergy production rate of 2891 MJ/ton-VS fed per day, which was two-fold higher compared to that achieved by one-stage AD.

1. Introduction

Food waste (FW) represents one of the most severe environmental problems faced by the society in the 21st century. FW accounts for ~ 45% of municipal solid waste and is considered a cross-cutting issue with multiple negative social, economic and environmental implications. For instance, the European Union generates annually ~ 88 Mt of FW, with an associated cost of 143bn € (FUSIONS, 2016). The global estimation for the FW generated in 2019 accounts to 931 Mt, which suggests that around 17% of the total amount of food produced globally may be wasted (Forbes et al., 2021). Nowadays, most of the FW is incinerated or landfilled, which entails prohibitive nutrient and energy losses and increases the total global greenhouse gas emissions by 8–10% (Mbow et al., 2019). In addition, FW is a major contributor to soil and water pollution. The United Nations 2030 Sustainable Development Goals have included a target to halve the global per capita FW generation at the retail and consumer levels. Likewise, the European Commission is committed to prevent FW generation along the entire food supply chain and to foster the creation of a circular bioeconomy in order to improve the sustainability and well-being of Europe and beyond. In this context, anaerobic digestion (AD) is nowadays the most cost-efficient and eco-friendly technology for recycling carbon, nutrients

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and energy from unavoidable FW, representing a cornerstone in the transition toward a circular bioeconomy (IEA, 2018; EBA, 2021).

Despite the potential of AD to recycle carbon and energy in the form of biogas, the widespread implementation of AD is nowadays limited by the poor robustness of conventional one-stage AD caused by the high hydrolysis and acidogenesis rate of FW, which typically lacks buffer capacity. Unlike one-stage AD configurations, two-stage AD processes allow buffering the acid stage and ensure optimal ecophysiological requirements for the different microbial communities involved, which may ultimately result in an enhancement of the overall process stability and bioenergy production (Shen et al., 2013; Baldi et al., 2019). However, and despite their potential superior performance, there is a scarce implementation of multi-stage systems on industrial or even pilot scales. Therefore, the development of innovative, sustainable and cost-effective strategies for improving AD performance and stability (maximizing bioenergy production) is crucial to ensure the competitiveness of the AD of FW in the context of a circular bioeconomy.

Two-stage AD is a sequential process in which hydrolysis and acidogenesis take place in a first reactor generating a hydrolyzed-acidified effluent that is further fed to a second bioreactor where acetogenesis and methanogenesis occurs. It is therefore reasonable to expect that the performance of the first stage may confer specific features to the acidogenic effluent and will ultimately impact the performance of the second stage. However, two-stage AD might be outperformed in terms of bioenergy production by its single-stage counterpart, particularly when high energy losses (production of hydrogen (H_2) and carbon dioxide (CO_2)) occur in the hydrolytic-acidogenic stage (Pipyn and Verstraete, 1981; Lindner et al., 2016; Feng et al., 2020). Therefore, it is imperative to optimize the first stage for the sake of achieving a superior global process performance in the two-stage AD of FW. In this context, lactate has been found to be the most appropriate substrate for methane formation compared to other acidogenic end-products such as butyrate or propionate (Pipyn and Verstraete, 1981; Wu et al., 2016; García-Depraect et al., 2020; Diaz-Cruces et al., 2020; Detman et al., 2021). From a bioenergetic viewpoint, lactate is the most energetically favorable acidogenic end-product for methanization (Pipyn and Verstraete, 1981). The use of acidified FW rich in lactate has indeed been found to mediate higher biogas production in comparison with unfermented FW (Daly et al., 2020, Guan et al., 2021). However, the knowledge of the design and operational conditions required to optimize the lactate type-fermentation of FW is still limited.

Herein, the effect of using an enriched lactate-producing consortium as inoculum and operational pH on the acidogenic fermentation of FW was investigated to elucidate how the prevailing culture pH and biocatalyst would affect the carbon and electron flow as well as the microbial community structure involved in lactate-based metabolic pathways. This study also aimed to assess via biochemical methane (CH_4) potential (BMP) tests to what extent the further acetogenic-methanogenic stage might be impacted when the acidified FW has already experienced different fermentation pathways including the lactate-type fermentation. Raw unfermented FW (mimicking one-stage AD) was also tested in parallel for the sake of comparison. Finally, an energy balance assessment was conducted as an attempt to systematically compare the one- and two-stage AD scenarios for fully exploiting the untapped energy potential of FW. This study provided new insights for driving the acidogenic FW fermentation towards a primary lactate-type fermentation and showed the feasibility and kinetic advantage of lactate-based two-stage AD of FW.

2. Materials and methods

2.1. Food waste

Real FW derived from a local restaurant at Valladolid (Spain) was collected and used as the substrate in this study. It mainly consisted of meat, fish, bread, cooked rice, vegetables such as lettuce, fried potatoes, tomato, onion, and oil. The FW was blended with tap water 20% (*w/w*) using a kitchen blender and stored at −20 °C. The physicochemical characteristics of the thawed FW mixture were as follows: pH 5.5, 430 g total chemical oxygen demand (COD)/L, 72 g soluble DQO/L, total solids (TS) 29.9% *w/w*, volatile solids (VS) 28.2% *w/w*, carbohydrates 38.3 % *w/w*, proteins 33.2% *w/w*, lipids 22.2% *w/w*, and ash 6.1% *w/w* (on a dry weight basis).

2.2. Set up and operational conditions of the hydrolytic-acidogenic stage

A series of batch fermentations were performed in duplicate to evaluate the effect of pH and biocatalyst on the hydrolysis-acidogenesis of FW. The experiments were carried out in a temperature-controlled room (36 ± 1) using two 1.25-L polyvinylchloride gas-tight bioreactors with a working volume of 0.7 L and magnetically stirred at ~ 300 rpm. Each bioreactor was equipped with a pH controller (EVopH-P5, BSV Electronic, Spain), sampling ports for the liquid and gas phase, and an in-house automatized gas flow meter which is based on the principle of liquid displacement. The fermentation conditions included i) uncontrolled pH with inoculum addition, ii) uncontrolled pH without inoculum addition, iii) controlled pH with inoculum addition and iv) controlled pH without inoculum addition. Operational pH was adjusted at 5.7 ± 0.1 using 6 M NaOH, while the initial pH for the uncontrolled pH condition was 6.7 due to the addition of 5 g/L sodium bicarbonate (buffering agent). Those selected fermentations were inoculated at 20% *v/v*. The acidogenic inoculum was a heat-shock (90 °C for 20 min) pretreated digestate derived from a pilot-scale anaerobic digester treating FW under mesophilic conditions. The TS content of all fermentations was initially adjusted to 10% using tap water. Gas and liquid samples were taken over 72 h to determine gas composition and metabolic end-products. At the end of the fermentation, the culture broth was recovered, characterized for VS content and organic acids profile, and preserved (for two weeks) at 4 °C to avoid any possible change in the organic acids produced prior BMP tests. Analyses performed before initiation of the BMP tests indicated that neither the VS content nor the organic acid profile of the acidogenic broths changed during this low temperature storage. Additionally, the microbial community structure of the acidogenic inoculum, the autochthonous microflora of the (frozen) FW, as well as of the hydrolytic-acidogenic process carried out at the different conditions tested was analyzed. The sampling points for microbial analysis were taken at different times, namely 31.5 (non-controlled pH with inoculum), 32.5 (controlled pH with inoculum) and 40 h (controlled pH without inoculum), which corresponded to the cultivation times near the maximum lactate uptake rate computed (see Supplementary information). Likewise, the reactor sample for microbial analysis in the self-fermentation conducted under uncontrolled pH conditions was drawn at 22.5 h, which corresponded to the cultivation time of maximum lactate accumulation rate.

2.3. Set up and operational conditions of the acetogenic-methanogenic stage

BMP tests were carried out as an attempt to evaluate the methanization of the four different acidogenic effluents harvested and of the raw unfermented FW. The tests were carried out in triplicate in 120-mL gas-tight glass serum flasks (50 mL working volume) incubated in an orbital shaker at 120 rpm and 36 ± 1 °C. Digestate from a pilot-scale anaerobic digester treating FW under mesophilic conditions was used as the methanogenic seed. This inoculum exhibited a VS/TS ratio of 0.6 and a slightly alkaline pH of ~ 7.8. The anaerobic sludge was preincubated for 7 days at 36 ± 1 °C to reduce the background (endogenous) biogas production and then amended with sodium bicarbonate (5 g/L). The food-to-microorganism (F/M) ratio (on VS basis) was adjusted to 0.25. Flasks were flushed with pure helium gas (Abello Linde, Barcelona, Spain) for 1 min to completely remove all residual oxygen from the

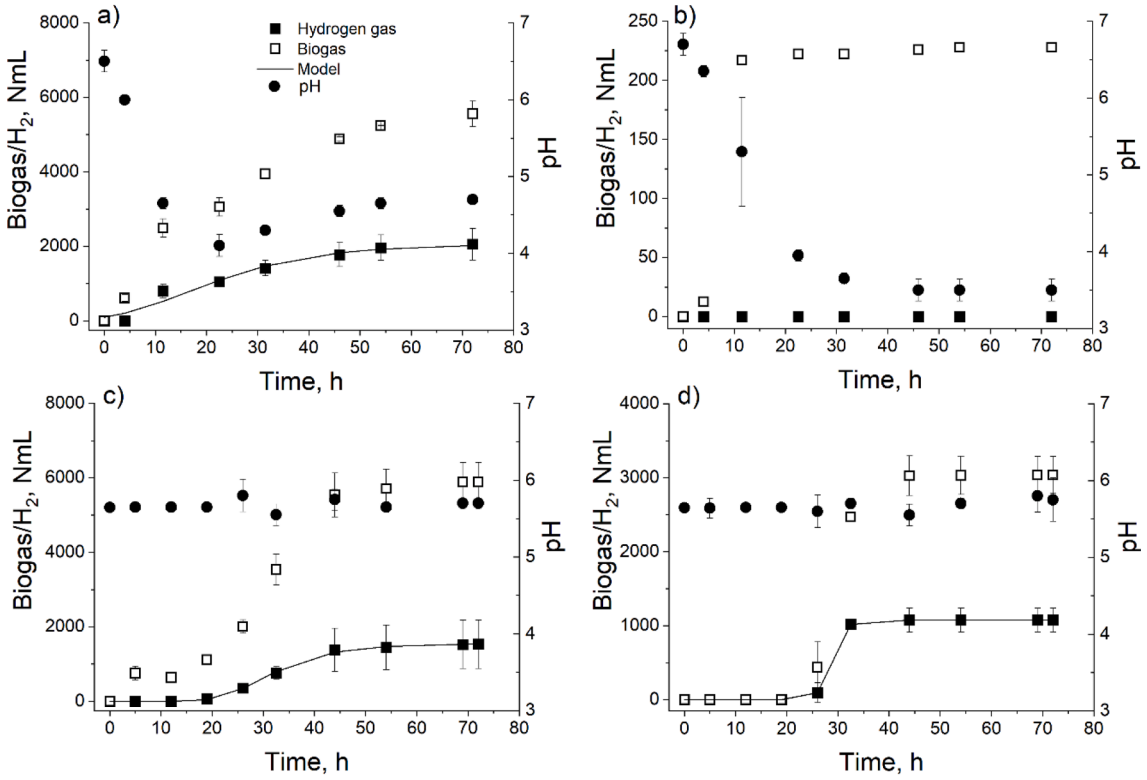

Fig. 1. Effect of the operational pH and inoculum addition on the profile of cumulative biogas/H_2 production during the batchwise acidogenesis of FW. a) pH non-controlled with inoculum addition, b) pH non-controlled without inoculum addition, c) controlled pH with inoculum, d) controlled pH without inoculum. Each condition was assessed in duplicate, and the data points in the graph correspond to the mean values and standard deviations (error bars). Solid lines represent the fitting trend for H_2 production using the modified Gompertz model (Diaz-Cruces et al., 2020).

headspace. The assays were incubated for 24 days, the incubation time when BMP curves plateaued. Blank tests with only inoculum were carried out in parallel to correct for the endogenous CH_4 production, while positive tests with cellulose pointed out the good quality of the methanogenic inoculum (318 ± 8 NmL CH_4/g VS). The CH_4 recovery rate (biodegradability) was estimated as the ratio between the experimental CH_4 yield and the theoretical CH_4 yield based on the structural characterization of the FW, according to Cheng and Liu (2012). Finally, an energy balance analysis was conducted on the basis of one ton of VS fed, the VS loss recorded during the first hydrolytic-acidogenic stage and the maximum volumetric H_2 and CH_4 production rates. Higher heating values of 12.74 kJ/NL and 35.16 kJ/NL were herein used for H_2 and CH_4, respectively (Diaz-Cruces et al., 2020).

2.4. Analytical procedures

Organic acid concentrations (formic, acetic, lactic, propionic, *iso*-butyric, butyric, *iso*-valeric, valeric, *iso*-caproic, hexanoic and heptanoic acid) were measured in an Alliance HPLC system (Waters e2695, USA) equipped with a UV–VIS detector (Alliance 2998 PDA, Waters, USA) set at 214 nm and a precolumn Micro-Guard Cation H + Refill Cartridge of 30 × 4.6 mm coupled with an Aminex HPX-87H chromatographic column (Bio Rad, USA). The column temperature was kept at 75 °C, while the eluent was 25 mM H_2SO_4 at a flow rate of 0.7 mL/min. A standard mix (Sigma-Aldrich part number CRM46975, USA) and sodium L-lactate (Sigma-Aldrich part number 71718, USA) were used for the calibration curves. Gas composition (H_2, CO_2, CH_4) was analyzed using a Varian CP-3800 gas chromatograph (Varian, USA) equipped with a thermal conductivity detector and a Varian CP-Molsieve 5A Capillary Column (15 m, 0.53 mm, 15 μm) interconnected with a Varian CP-PoreBOND Q Capillary Column (25 m, 0.53 mm, 10 μm), and calibrated using certified standard gas mixtures of known composition (70.0% H_2 and 30.0 CO_2; 70.53% CH_4, 24.0% CO_2, 2.99% N_2, 2.0% H_2S, 0.49% O_2). Ultra-pure helium gas was used as the carrier gas at 13 mL/min. Cumulative CH_4 production was estimated by manometric and gas-chromatographic methods, as reported elsewhere (García-Depraect et al., 2022). CH_4 yields were calculated according to Diaz-Cruces et al. (2020). Total carbohydrates were analyzed by the phenol–sulfuric method, while the protein content was estimated from total Kjeldahl nitrogen (TKN) determination using a conversion factor of 6.25. The lipid content determination protocol of the Regional Service for Agri-food Research and Development (SERIDA, Spain) was used to measure the lipid content of the blended FW. COD, pH, solids and TKN were measured according to standard methods (APHA, 2005). All reagents used herein were of analytical grade.

Microbial community structure was determined by amplifying the V3-V4 region of the 16S rRNA gene using the primer set 341F-805R, according to the 16S Metagenomic Sequencing Library Illumina15044223 B protocol (Klindworth et al., 2013). The sequencing data obtained were analysed into the QIIME2 platform (Bolyen et al., 2019). Clean amplicon sequencing variants (ASVs) were annotated against NCBI 16S rRNA database version 2021 at a 97% similarity, while SILVA database v.138 was used for those ASVs assigned with < 97% identity. Data was normalized using rarefaction technique from Phyloseq R package to perform alpha diversity analysis (Weiss et al., 2017). Shannon-Wiener and Simpson (1-D) diversity indices were calculated using Past software (version 4.09).

3. Results and discussion

3.1. Operational performance of the hydrolytic-acidogenic stage

The influence of both inoculum addition and pH control on the hydrolysis-acidogenesis of FW was initially evaluated in terms of the

Table 1
Interplay effect between operational pH and biocatalyst on some process performance features recorded during the hydrolytic-acidogenic stage.

Condition	Uncontrolled pH & with inoculation	Uncontrolled pH & without inoculation	Controlled pH & with inoculation	Controlled pH & without inoculation
H_2 yield (NmL/g VS fed)	31.2 ± 6.4	–	23.2 ± 9.9	16.3 ± 2.5
Peak H_2 (% *v/v*)	53.4 ± 3.7	N.D.	46.1 ± 27.9	40.1 ± 9.0
Peak CO_2 (% *v/v*)	46.6 ± 3.7	100 ± 0	53.7 ± 27.9	59.9 ± 9.0
CH_4 (% *v/v*)	N.D.	N.D.	N.D.	N.D.
Total concentration of C released as CO_2 gas (g/L)	2.7 ± 0.1	0.2 ± 0.2	3.3 ± 0.1	1.5 ± 0.3
[a] *P* (NmL H_2)	2062.8	–	1540.7	1075.0
[a] *Rm* (NmL H_2/h)	52.9	–	71.8	233.4
[a] λ (h)	1.7	–	21.3	25.8
R^2	0.9861	–	0.9990	0.9999
[b] Total concentration of C measured as organic acids (g/L)	7.9 ± 0.5	9.7 ± 0.02	9.9 ± 0.3	10.5 ± 1.8
[b] Organic acids concentration (g CODeq./L)	25.5 ± 1.7	26.6 ± 0.2	31.1 ± 0.04	32.9 ± 4.9
[b] Organic acids yield (mg CODeq/g VS fed)	271.1 ± 17.9	283.5 ± 2.1	331.6 ± 0.5	350.4 ± 52.7
[c] Acidification rate (%)	17.8 ± 1.2	18.6 ± 0.1	21.7 ± 0.03	22.9 ± 3.4
[d] Electron equivalents disposed as H_2 gas (e^- meq)	261.8 ± 53.6	0	195.0 ± 83.0	137.1 ± 20.6
Final VS removal (%)	32.7 ± 7.2	15.9 ± 0.2	50.9 ± 3.6	42.6 ± 6.0

N.D.: Not detected.
[a] Kinetics parameters obtained from the modified Gompertz model; *P*: maximum cumulative H_2 production, *Rm*: maximum H_2 production rate, λ: lag phase.
[b] Organic acids measured at the end of the hydrolytic-acidogenic stage.
[c] Acidification degree was estimated as the ratio (expressed in %) of the COD equivalent of organic acids measured at the end of the fermentation and the total COD of the substrate.
[d] 1 mmol H_2 produced is equivalent to 2 e^- meq.

acidogenic off-gas production (Fig. 1a–d, Table 1). In this context, both studied factors had a clear effect on the extent and rate of biogas production and its composition. A higher cumulative production of biogas of up to 5.9 ± 0.5 NL and of H_2 of up to 2.1 ± 0.4 NL (equivalent to 31.2 ± 6.4 NmL/g VS added) was computed in the inoculated reactors regardless of the pH condition (Fig. 1). The H_2 content in the acidogenic off-gas peaked at 46–53% and was higher in the inoculum-aided fermentations compared with the self-fermentations, while no CH_4 was detected under the conditions studied (Table 1). Remarkably, the self-fermentation (without inoculum addition) of FW allowed for a lower loss of carbon and reducing equivalents as gaseous CO_2 and H_2, respectively, compared with the inoculum-assisted fermentations

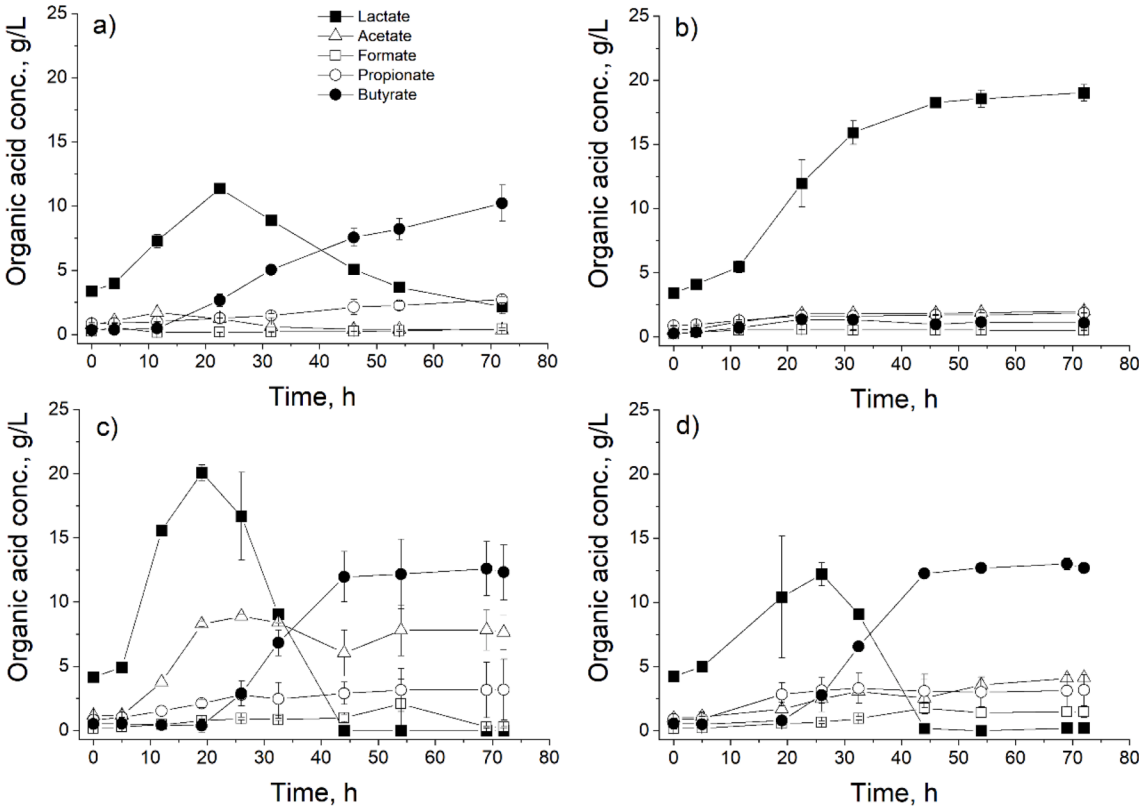

Fig. 2. Effect of the operational pH and inoculum addition on the profile of organic acids during the batchwise acidogenesis of FW. a) pH non-controlled with inoculum addition, b) pH non-controlled without inoculum addition, c) controlled pH (at 5.7 ± 0.1) with inoculum, d) controlled pH (at 5.7 ± 0.1) without inoculum. Each condition was assessed in duplicate, and the data points in the graph correspond to the mean values and standard deviations (error bars).

regardless of the pH conditions (Table 1). The lowest biogas production (0.27 NL) was observed under non-controlled pH and without inoculum addition, which indeed prevented the onset of H_2 production (Fig. 1b). This carbon and energy saving condition seems a favorable scenario to enhance CH_4 production in the second methanogenic stage, as more energy-rich acidogenic effluents with reduced compounds such as lactate were obtained (Chakraborty et al., 2022; García-Depraect et al., 2020).

At this point it should be highlighted that the ultimate goal of this study was to enhance the CH_4 production kinetics from FW by steering the lactate-type metabolic pathway during hydrolysis-acidogenesis rather than the production of biogenic H_2. Nevertheless, it was interesting to observe that the bioconversion of FW into H_2 proceeded through the lactate-driven dark fermentation process, even despite the operational pH decreased down to ~ 4.2 (Fig. 1a and 2a). Similar results have been reported previously by Kim et al. (2012). Nonetheless, the hydrogenogenic activity was evident when the initial pH was near to neutral (~6.7) with the aid of a pH buffer and totally inhibited when FW with no pH adjustment (initial pH 5.3–5.5) was used, as discussed below.

The differences in the production of biogas and H_2 herein observed could be explained by considering two aspects. On one hand, the addition of an inoculum harboring vigorous acidogenic microorganisms, including H_2-producing bacteria (as will be discussed in Section 3.1.2), boosted the acidogenic off-gas production, as previously observed by Favaro et al. (2013). The VS reduction efficiency was always higher in the inoculated fermenters (32.7 vs. 15.9% and 50.9 vs. 42.6% for uncontrolled- and controlled-pH conditions, respectively; Table 1), indicating high hydrolytic-acidogenic activity. Such VS destruction values were similar to those previously found during the acidogenesis of FW, which have been reported to range between 26 and 50% (Kim et al., 2009; Moon et al., 2015). On the other hand, it is well established that the pH of the fermentation broth is one of the major influencing factors in the performance of acidogenic systems (Daly et al., 2020; Infantes et al., 2011; Sarkar et al., 2021), and thus it also affected the extent and rate of the evolved biogas (Table 1).

The acidogenic reactors operated under controlled pH conditions kept a constant pH value of ≈ 5.7, with slight deviations of ± 0.1 (Fig. 1a, c), which supported the growth and maintenance of hydrolytic-acidogenic bacteria in addition to H_2 production (García-Depraect et al., 2021). In the uncontrolled pH tests with and without inoculum addition, the initial pH of the fermentation broth experienced a rapid drop during the first 24 h of fermentation, from 6.7 to pH values close to 4.0. Afterwards, the pH tended to increase up to 4.7 in the inoculum-assisted fermentations but, conversely, it went down to 3.5 in the self-fermentations (Fig. 1a, b). The sharp decline in pH was attributed to the accumulation of lactate (pKa 3.8) mediated by the vigorous activity of lactic acid bacteria (LAB) (Yang et al., 2022). The slightly increase in pH was attributed to the consumption of lactate, as observed elsewhere (Blanco et al., 2019), mainly by lactate-consuming H_2-producing bacteria (García-Depraect et al., 2021). Little or no biogas production occurred when no pH buffering agent (sodium bicarbonate) was added even in inoculated fermenters (data not shown), pointing out that both the initial and operational pH impacted the metabolic flux. Therefore, the production of biogas or H_2 herein recorded cannot be explained only by considering the bioaugmentation of specialized microorganisms but its interplay with the operational pH.

3.1.1. *Metabolic end-products distribution: The key role of lactate during the acidogenesis of FW*

The fermentation product spectra recorded during the batchwise acidogenesis of FW are represented in Fig. 2. Both the inoculum addition and pH control influenced the prevailing acidogenic metabolic pathways. Hence, lactate and butyrate were the two major organic acids detected at the end of the batch tests, with concentrations as high as 19.1 (0.2 g/g VS fed) and 13.0 g/L (0.14 g/g VS fed), respectively. However, there was a trade-off between such end-products: the higher the butyrate concentration, the lower the lactate titer and vice versa. The time of incubation and the condition tested (i.e., prevailing biocatalyst and pH) determined whether lactate or butyrate was the major end-product. Acetate, formate and propionate were also detected, and their concentrations fluctuated between 0.4 and 8.9 g/L, 0.2 and 2.1 g/L, and 0.9 and 3.2 g/L, respectively, depending on the condition tested. This indicated the presence, yet to a minor extent, of other side acidogenic metabolic pathways such as mixed-, acetic- and propionic-type fermentation pathways (Zhou et al., 2018). The relatively high acetate concentrations recorded (particularly in fermentations performed under pH-controlled conditions) could be also evidence of homoacetogenesis, explaining the low H_2 production recorded. At the end of the test, the self-fermentation with pH control exhibited a slightly higher organic acid yield (350.4 ± 52.7 mg CODeq/g VS fed with an associated acidification rate of ≈ 23%) in comparison with the other conditions tested (Table 1).

Overall, two different metabolic pathways for lactate were observed, i) the fermentation of carbohydrates into lactate via homolactic, heterolactic, or Bifidus pathway (García-Depraect and León-Becerril, 2018) and ii) the fermentation of lactate into butyrate with the coproduction of H_2 and CO_2 (Diez-Gonzalez et al., 1995; Matsumoto and Nishimura, 2007). The oxidation of formate catalyzed by the enzyme formate hydrogen lyase (FHL) might have also contributed to produce H_2, although to a lesser extent than the lactate-driven H_2 production. Interestingly, all the conditions tested diverted the metabolic flux towards the accumulation of lactate during first 20 h of culture, and afterwards towards its metabolization via the butyrate-type fermentation (Fig. 2a,c,d), except for the FW fermentation carried out with no addition of inoculum and without pH control. This latter condition resulted in a prominent build-up of lactate during the first 48 h of culture, which remained essentially constant until the end of the fermentation (Fig. 2b). Interestingly, the test carried out under pH-controlled conditions with the addition of inoculum showed the fastest lactate production and consumption rates, peaking at 1.5 and 1.2 g lactate/L-h, respectively, which corresponded to the highest VS removal, as discussed earlier. A higher FW-to-lactate bioconversion is typically linked to a higher hydrolytic activity (Peinemann et al., 2019), but hydrolysis and consequently LAB growth and lactate production are commonly impaired when uncontrolled pH decreases below pH 4 (Tang et al., 2017). The second highest lactate production rate (0.59 g lactate/L-h) was found when indigenous microorganisms grown under uncontrolled pH conditions, showing good autochthonous microbial activity for the spontaneous lactate fermentation. Comparatively, the other combinations applied entailed, in average, lower lactate production/consumption rates (see Supplementary information).

Accordingly, the sequential two-stage lactate fermentation has been reported elsewhere in studies aimed at producing H_2 via dark fermentation (Asunis et al., 2019; Blanco et al., 2019; García-Depraect et al., 2019; García-Depraect and León-Becerril, 2018). From a microbiological point of view, the metabolic patterns observed herein could be explained by reaction time-dependent successions of bacterial communities. It has been shown that LAB produce lactate as the main end-product from the acidogenic breakdown of carbohydrates during a first fermentation step, and the produced lactate is then metabolized by butyrogenic bacteria such as *Clostridium butyricum* and related species during a secondary lactate fermentation (García-Depraect et al., 2019). The balance between such microbial groups (i.e., LAB and butyrogenic bacteria) is typically pH-dependent (García-Depraect et al., 2021). Compared to spontaneous lactic acid fermentation, bioaugmentation of LAB has been proven as a useful strategy to achieve enhanced lactate production yields (Zhang et al., 2021). However, the presence of the autochthonous communities in non-sterile fermentations may influence the fermentation outcome even when adding an external workhorse (Peinemann et al., 2019). Accordingly, it could be inferred that both the acidogenic microbial consortium used in this study and the indigenous microflora present in the FW harbored the metabolic potential to

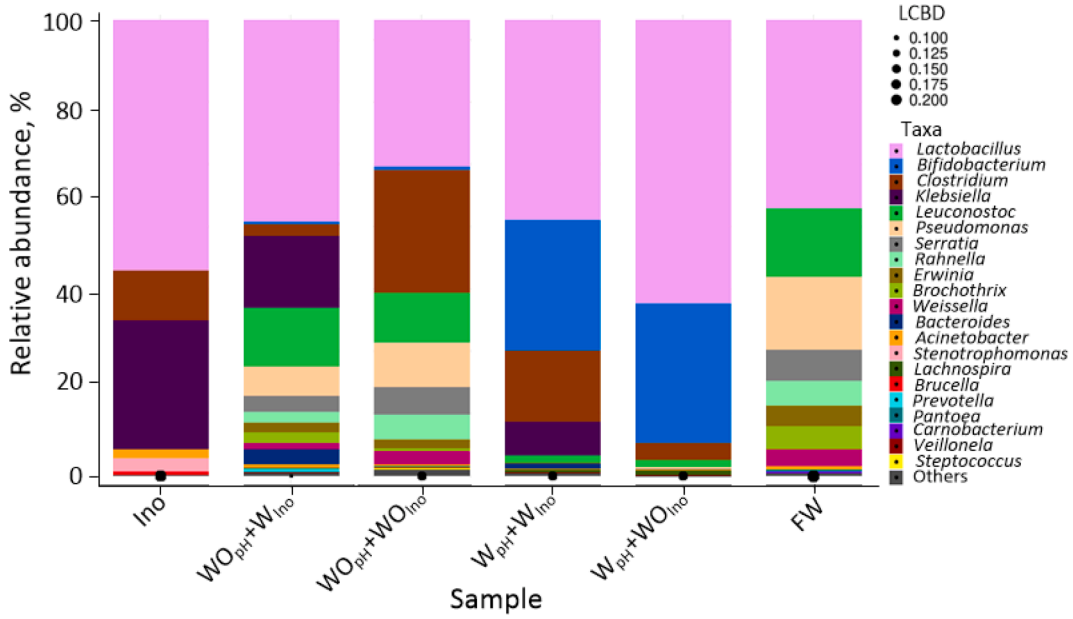

Fig. 3. Observed microbial diversity (at genus level) in the inoculum (Ino), the substrate (FW) and during the hydrolysis-acidogenesis of FW performed without pH control and with inoculum addition (WO$_{pH}$ + W$_{Ino}$), without pH control and without inoculum addition (WO$_{pH}$ + WO$_{Ino}$), with pH control and with inoculum addition (W$_{pH}$ + W$_{Ino}$), and with pH control and without inoculum addition (W$_{pH}$ + WO$_{Ino}$). Local contribution to beta-diversity (LCBD) stands for a dissimilarity coefficient; the higher the LCBD, the higher the dissimilarity among the analyzed samples.

perform the sequential two-stage lactate fermentation. In this context, the accumulation of lactate (76.3 ± 1.9% selectivity) was favored when the secondary lactate fermentation was prevented, a fact that seems challenging but was achieved herein by spontaneously fermenting FW under non-controlled pH conditions. Tailored environmental and operational conditions, such as a low pH and/or short reaction time, have been also proven to increase lactate selectivity (Feng et al., 2018; García-Depraect et al., 2020).

3.1.2. Acidogenic microbial community analysis

Bridging microbial insights with process engineering helps to attain a better understanding of the hydrolysis-acidogenesis of FW. The microbial diversity of the inoculum included genera belonging to *Lactobacillus*, *Klebsiella*, *Clostridium*, *Stenotrophomonas*, *Acinetobacter*, with relative abundances of 55.2, 28.0, 10.9, 3.0 and 1.8%, respectively, together accounting for > 99% of the total community (Fig. 3). Other satellite bacteria with a relative low abundance (<1.0%) were *Brucella*, *Curvibacter*, *Pseudomonas*, and *Methylomicrobium*. Notably, *Pseudomonas* was the only microorganism amongst such subdominant bacteria that was detected at high relative abundances (>1%) in the hydrolytic-acidogenic process, particularly when pH was not controlled.

Pseudomonas was part of the autochthonous microbiota present in the FW employed (16% relative abundance). In the FW, *Lactobacillus* was the most dominant bacterial genus, accounting for 41.1% of the total reads, which was a reasonable result since FW is a good growth medium for this LAB, which indeed is well-known for being among the most dominant spoilage microorganisms in the spontaneous fermentation of carbohydrates-rich substrates such as FW (Im et al., 2020; Wu et al., 2018). The microbiota of the substrate also harbored bacterial genera belonging to *Leuconostoc* (15.0%), *Serratia* (7.0%), *Rahnella* (5.4%), *Brochothrix* (5.1%), *Erwinia* (4.4%) and *Weissella* (3.5%). Interestingly, all such bacteria were detected during the hydrolytic-acidogenic stage, particularly in tests conducted without pH control, with relative abundances lower than 10% except in the case of *Leuconostoc* (10.8–12.7%). That prevalence of autochthonous microbiota evidenced that the prevailing bacteria during the hydrolytic-acidogenic stage might originate, at least partially, from the FW itself, thereby leading to an open fermentation system.

It was also found that regardless of the inoculation of the hydrolytic-acidogenic fermenter, pH-uncontrolled conditions led to a relatively higher diversity and evenness of the communities compared to those prevailing under controlled pH, as evidenced by the Shannon and Simpson diversity indices (see Supplementary materials). Therefore, the controlled and uncontrolled pH regime determined in some extent clustering in the microbial communities, as supported by the hierarchical clustering analysis (see Supplementary materials). Under non-controlled pH conditions, the addition of inoculum resulted in a microbial community harboring *Lactobacillus* (43.9%), *Klebsiella* (15.7%), *Leuconostoc* (12.7%), *Pseudomonas* (6.6%), *Serratia* (3.4%), *Bacteroides* (3.2%), *Clostridium* (2.5%), *Rahnella* (2.4%), *Brochothrix* (2.2%), *Erwinia* (2.2%), and *Weissella* (1.6%). Comparatively, the microbial community in the fermenter was still dominated by *Lactobacillus* (32.2%) in the absence of inoculum, followed by *Clostridium* (26.9%), *Leuconostoc* (12.7%), *Pseudomonas* (9.9%), *Serratia* (6.0%), *Rahnella* (5.4%), *Weissella* (2.7%), and *Erwinia* (1.7%). The acid-tolerance mechanisms against low pH stress developed by certain acidogenic bacteria may explain in part the relatively higher species diversity found herein under non-adjusted pH conditions, characterized by experiencing a sharp pH drop down to 3.5–4.7 mainly due to lactate accumulation. In fact, some acidogenic bacteria such as *Lactobacillus* can exhibit a relatively high microbial activity at low pH of 4.0 (Wu et al., 2015). Obviously, the prevailing pH widely varied under non-controlled pH conditions in comparison with pH-controlled conditions, and such dynamic pH microenvironment (broader pH range) might enable the growth of different types of microorganisms, which represents another possible explanation for the higher microbial diversity observed.

Interestingly, the dominant bacteria at a fixed pH of 5.7 ± 0.1 were *Lactobacillus*, *Bifidobacterium* and, in a minor extent, *Clostridium*, representing 88.1 and 96.6% of the total community in the inoculum-aided fermentation and the self-fermentation, respectively (Fig. 3). Unlike *Lactobacillus*, *Bifidobacterium* is typically more sensitive to low pH stress (Ventura et al. 2011), thus it tends to growth better under slightly acidic environments (Wu et al., 2015), explaining why it became dominant at adjusted pH of 5.7 ± 0.1 while its relative abundance remained below 1.0% when pH dropped down to 3.5–4.7. *Klebsiella* was also found to be an accompanying bacterium but only in inoculated fermentations.

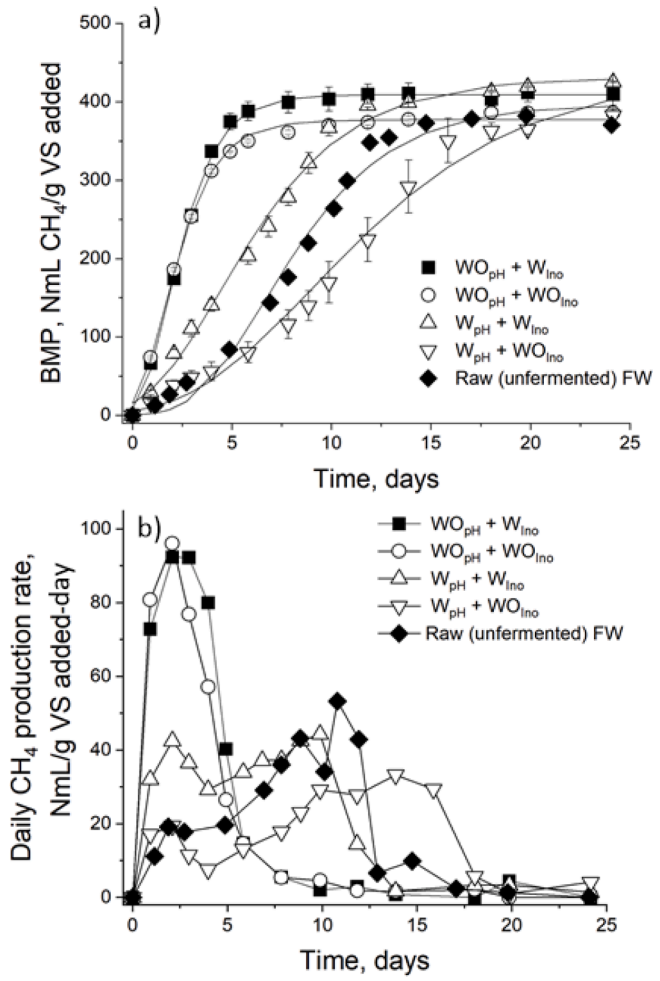

Fig. 4. Time course of the accumulated methane produced (a) and daily methane production rate (b) for the raw unfermented FW (control) and the four different acidified FW effluents. Solid lines in figure (a) represent the fitting trends using the modified Gompertz model (Diaz-Cruces et al., 2020).

In general, both the off-gas production and the metabolic spectra observed during the hydrolytic-acidogenic stage were clearly endorsed by the microbial communities identified. Particularly, the production of lactate was evidently explained by the primary dominance of *Lactobacillus*, the lactate producer par excellence. Besides, *Leuconostoc* is a heterofermentative LAB that can produce acetate/ethanol and CO_2 in addition to lactate and is commonly found in FW fermentations (Wu et al., 2018, 2015). Likewise, the *Bifidobacterium* genus is often regarded as a member of LAB with saccharolytic activity that can perform the Bifidus pathway, thus producing 1 mol of lactate and 1.5 mol of acetate from 1 mol of glucose (Cheng et al., 2008). Meanwhile, *Klebsiella* and *Clostridium* are well-known H_2 producers, fulfilling the H_2 production function. It has been previously reported that *K. pneumoniae* is equipped with the FHL complex, which is the main responsible of its H_2 production capacity (Jung et al., 2014). On the other hand, some strains of *Clostridium* such as *C. butyricum* and *C. tyrobutyricum* can consume lactate (commonly along with acetate) to produce H_2 with concomitant butyrate and CO_2 production (Matsumoto and Nishimura, 2007), thus interacting with LAB by cross-feeding interactions (García-Depraect et al., 2021). Finally, it is worth noting that satellite bacteria could have a significant role in the acidogenic process despite their lower abundance. For instance, *Pseudomonas* is a facultative anaerobe commonly detected in anaerobic fermenters wherein it can help strict anaerobes to thrive by consuming residual oxygen and by breaking down complex organic substrates (Hung et al., 2011).

3.2. Biochemical methane production tests: Assessing single- and two-stage AD

The different conditions applied in the hydrolytic-acidogenic stage induced a markedly effect on the subsequent production of CH_4, especially in its kinetics (Fig. 4). Overall, the CH_4 yields achieved after 24 days of incubation in the two-stage AD processes ranged from 382.6 to 425.3 NmL/g VS added depending on the prevailing conditions experienced during hydrolysis-acidogenesis. The highest CH_4 yield recorded (corresponding to 82.3% CH_4 recovery rate) was attained by inoculating the hydrolytic-acidogenic reactor while keeping the pH constant, whereas self-fermentation resulted in the lowest CH_4 conversion (with an associated CH_4 recovery rate of 74.2%) regardless of the pH control strategy implemented (Fig. 4a). Comparatively, raw unfermented FW supported 370.6 ± 1.9 NmL CH_4/g VS added (71.2% CH_4 recovery rate), which was in close agreement with the typical CH_4 yields (200–500 NmL CH_4/g VS) reported in literature for the one-stage AD of FW (Chakraborty et al., 2022). In terms of CH_4 yield, the two-stage AD configuration outperformed its one-stage counterpart by ≈ 13% under optimal conditions. This enhancement in CH_4 production by physically separating hydrolysis-acidogenesis from acetogenesis-methanogenesis lies within the values reported elsewhere for two-stage AD systems. For instance, Baldi et al. (2019) conducted a comparative study of one- and two-stage AD bioreactors continuously treating FW under mesophilic conditions and reported an enhancement of 6.0% in CH_4 production by implementing a sequential process. Similarly, De Gioannis et al. (2017) found that a two-stage process recovered 19% more CH_4 from FW than one-stage AD.

The different conditions imposed in the hydrolytic-acidogenic stage also supported different CH_4 production kinetics (Fig. 4b), indicating that the first hydrolysis-acidogenic step must be optimized in order to mediate an enhanced bioenergy production rate. As shown in Table 2, the maximum daily specific CH_4 production rate (*Rm*) was estimated by adjusting the CH_4 yield data computed over time to the modified Gompertz model (Diaz-Cruces et al., 2020), which correlated the experimental data more accurately than the first-order kinetic model or the logistic model (data not shown). The methanization of raw FW resulted in a maximum CH_4 production rate of 42 NmL/g VS added-day, which was very similar to the 43.3 NmL/g VS added-day mediated by the fermentation broth resulting from inoculated acidogenic tests with pH control. Interestingly, the two highest *Rm* values (109.9 and 97.6

Table 2
Modified Gompertz model parameters obtained for the different conditions tested.

Condition	pH control & inoculation	pH control & without inoculation	Without pH control & inoculation	Without pH control & without inoculation	Raw unfermented FW
BMP∞ (NmL CH_4/g VS added)	430.6	438.6	408.7	376.9	397.2
Rm (NmL CH_4 g/VS added-d)	43.3	26.5	109.9	97.6	42.0
λ (day)	0.8	2.9	0.5	0.2	3.2
R^2	0.9997	0.9937	0.9993	0.9983	0.9946

BMP∞: ultimate methane production potential; *Rm*: maximum daily methane production rate; λ: lag phase.

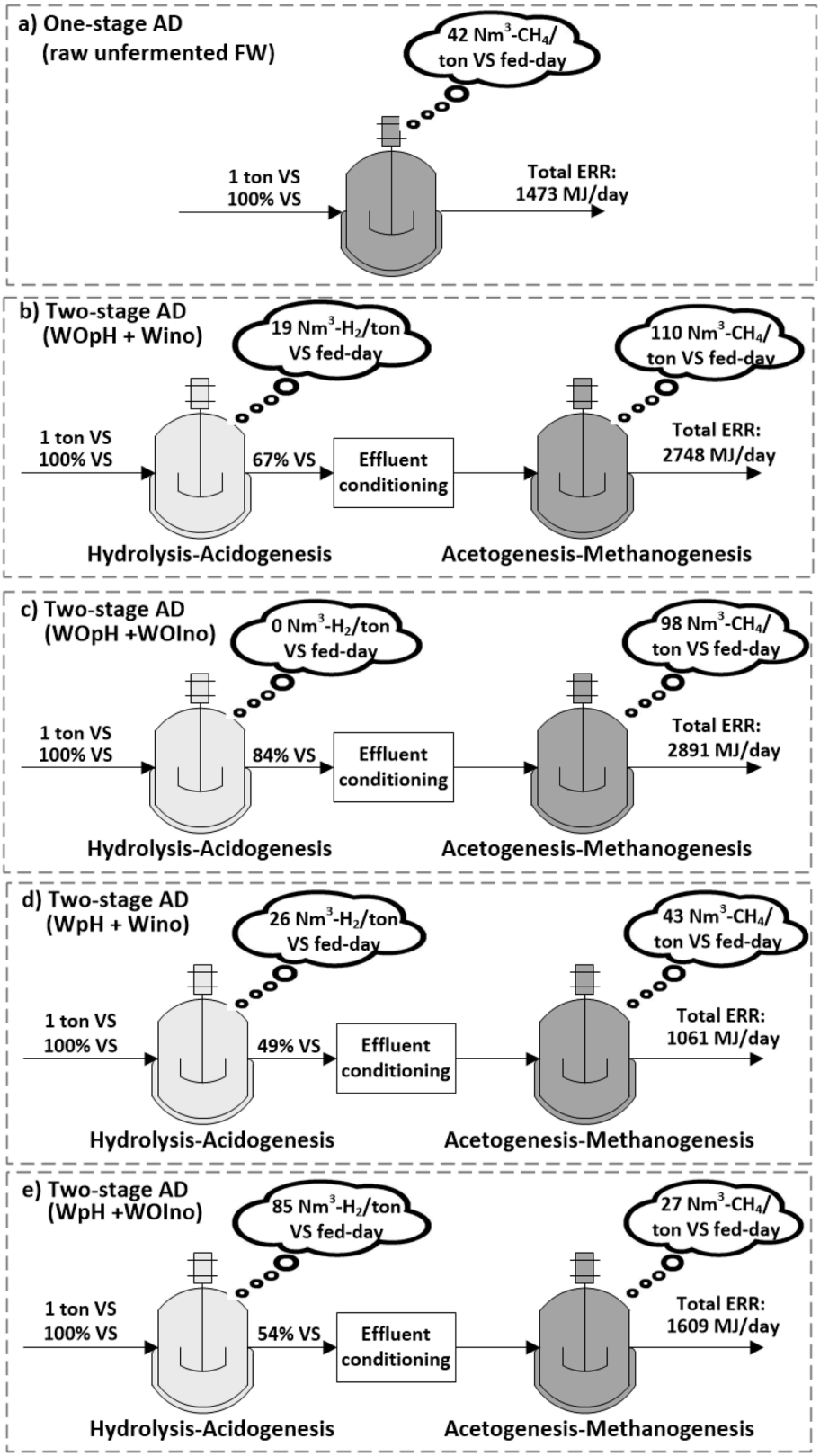

Fig. 5. Energy balance for the one-stage and two-stage AD scenarios. ERR: energy recovery rate.

NmL CH_4 g/VS added-day) were recorded using the fermentation broths derived from the hydrolytic-acidogenic stage conducted without pH control regardless of the initial inoculation strategy. The higher *Rm* recorded with the lactate-rich acidogenic broth (self-fermentation without pH control) could be explained by the fact that the acidogenic stage dominated by lactate accelerated the hydrolysis of FW while conserving more energy in the substrate (only 15.9% VS loss) to be methanized. From a bioenergetic point of view, acetogenic bacteria and methanogenic archaea can obtain more energy when lactate is the acidogenic end-product. Primary lactate-type fermentation entails a

value of Gibbs free energy change ($\Delta G^{0\prime}$) of −65.8 kJ (at standard physiological conditions) per mol of CH_4 produced, which is more negative than those from butyric-type fermentation (–32.6 kJ), acetic-type fermentation (-31 kJ), propionic-type fermentation (–32.1 kJ), and ethanol-type fermentation (-59.4 kJ) (Pipyn and Verstraete, 1981). Furthermore, the anaerobic oxidation of lactate produces acetate, H_2 and CO_2, which are immediate CH_4 precursors that can be simultaneously and rapidly metabolized by aceticlastic and hydrogenotrophic methanogens, respectively. This might ultimately foster syntrophic methanogenesis.

At this point it is worth noting that the corresponding final pH during the BMP tests remained at 7.5 ± 0.1 (Table 2). Such pH values are conducive for methanogenesis (De Gioannis et al., 2017), while no organic acids accumulation was detected at the end of all BMP tests (data not shown), implying high syntrophism among microorganisms. Unlike lactate oxidation, lactate accumulation may onset the formation of propionate at high partial H_2 pressures (>0.1 kPa) (Wu et al., 2016), however, no accumulation of propionate was here detected. Indeed, there was no evidence of inhibition in the CH_4 production evolution in preliminary BMP experiments carried out with acidified FW rich in propionate (7.9 ± 1.4 g/L) (data not shown). The butyrate-rich acidogenic effluent obtained from inoculated fermentations with pH control (32.7% VS loss) also underwent faster methanization, suggesting that syntrophic butyrate oxidizers and acetate- and H_2-consuming methanogens were extremely active, thus helping to maintain the H_2 partial pressure low. Contrarily, the methanization of the acidogenic effluent originated from the self-fermentation with controlled pH resulted in the lowest CH_4 production rate, which is a sign of inefficiency in the transformation of organic matter to CH_4 by acetogens and methanogens, likely due to an imbalance in the trophic microbial groups involved. However, it is not clear which factor was the rate limiting step in the methanization of such acidified FW sample.

3.3. Bioenergy production yield and rate

A rational comparison between the one-stage and two-stage AD of FW was performed considering the H_2 and CH_4 production yields and rates observed and the corresponding VS losses recorded during the hydrolytic-acidogenic stage. The highest energy production yield of 11.4 ± 0.04 MJ/ton VS added was achieved by digesting the lactate-rich acidogenic effluent, which was up to ~ 35% higher than those yielded by the other two-stage scenarios. However, the one-stage AD of FW yielded 13.0 MJ/ton VS added, which was 12.3–43.3% higher than those attained in the two-phase configuration. This means that neither the superior CH_4 yield achieved through the two-stage approach in comparison with that supported by the conventional one-stage AD, nor the recovery of extra energy as H_2 gas offset the VS loss occurred in the first stage, even when VS losses amounted only 16% of the initial VS content of FW. Bioenergy production in the form of H_2 accounted for 2.8–4.0% of the total energy, which is in line with H_2 productions previously reported in literature (De Gioannis et al., 2017). Based on the properties of the real FW herein employed, the one- and two-stage AD configurations tested yielded up to 4.4 and 3.9 MJ per each ton of FW digested, respectively.

However, the AD of FW through an adequate two-stage process brought about clear kinetics advantages to the process, which resulted in maximum global bioenergy production rates between 1061 and 2890 MJ/ton VS fed-day (Fig. 5), corresponding to the range of 359 to 978 MJ/ton FW fed-day. The highest energy production rate estimated was recorded in the two-stage AD of the lactate-rich acidified FW, despite the share of H_2 in this case was zero. The two-stage AD using the effluent derived from the hydrolytic-acidogenic stage performed with inoculum but without pH control resulted in a similar high bioenergy production rate (2748 MJ/ton VS fed-day or 923 MJ/ton FW fed-day), for which the H_2 productivity only accounted for 8.9% of the total energy production rate. Such high specific energy production rates were almost twofold higher than the one achieved in the one-stage process (1473.2 MJ/ton VS fed-day or 498.5 MJ/ton FW fed-day) (Fig. 5). The two-stage AD of the other two acidogenic broths resulted in slightly higher (9.2%) or even lower (28.0%) energy production rates compared to that of the conventional one-stage AD plant, notwithstanding the H_2 share amounted to 31.4 and 62.7%, respectively, of the total energy production rate.

3.4. Significance of the experimental data and future research

Nowadays, the engineering of two-stage AD processes for the treatment of organic waste such as FW with concomitant recovery of renewable bioenergy is gaining attention. The present study confirmed that the performance of the first hydrolytic-acidogenic stage could influence both the CH_4 production rate and yield. This study also showed that CH_4 production should be prioritized over H_2 production in a two-stage AD process to maximize the overall bioenergy production. In this regard, this study revealed the potential to attain superior bioenergy production rates compared to the traditional one-stage scheme by modulating the acidogenic fermentation pathways towards a primary lactate-type fermentation. In practical aspects, higher CH_4 production turnovers imply lower retention times or higher FW treatment capacities in kinetics terms, which ultimately means lower reactor volumes and reduced capital costs. In this context, long-term continuous operation of lactate-based two-stage AD will be required to optimize the process and fully exploit the untapped energy potential of FW. A large-demo scale study is also required to assess the economic and technical viability of the lactate-based two-stage AD of FW proposed herein.

4. Conclusions

This study evidenced that a lactate-based two-stage AD process doubled the bioenergy recovery rate from FW compared to one-stage AD. Additionally, this study showed that lactate metabolism has a key role in the hydrolysis-acidogenesis of FW and demonstrated that both the biocatalyst and culture pH are critical factors governing its fate which ultimately affects the methanization outcome. Particularly, lactate as the major acidogenic end-product was only achievable in the self-fermentation without pH control. Such tailored lactate-type fermentation solubilized particulate organics while saving carbon and reducing equivalents. Contrarily, lactate consumption by butyrogenic bacteria reduced the potentially available energy for syntrophic methanogenesis.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2022.127358.

References

APHA, 2005. Standard Methods for the Examination of Water and Wastewater.

Asunis, F., de Gioannis, G., Isipato, M., Muntoni, A., Polettini, A., Pomi, R., Rossi, A., Spiga, D., 2019. Control of fermentation duration and pH to orient biochemicals and biofuels production from cheese whey. Bioresour. Technol. 289, 121722 https://doi.org/10.1016/j.biortech.2019.121722.

Baldi, F., Pecorini, I., Iannelli, R., 2019. Comparison of single-stage and two-stage anaerobic co-digestion of food waste and activated sludge for hydrogen and methane production. Renew. Energy 143, 1755–1765. https://doi.org/10.1016/j.renene.2019.05.122.

Blanco, V.M.C., Oliveira, G.H.D., Zaiat, M., 2019. Dark fermentative biohydrogen production from synthetic cheese whey in an anaerobic structured-bed reactor: Performance evaluation and kinetic modeling. Renew. Energy 139, 1310–1319. https://doi.org/10.1016/j.renene.2019.03.029.

Bolyen, E., Rideout, J.R., Dillon, M.R., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37 https://doi.org/10.1038/s41587-019-0209-9.

Chakraborty, D., Karthikeyan, O.P., Selvam, A., Palani, S.G., Ghangrekar, M.M., Wong, J. W.C., 2022. Two-phase anaerobic digestion of food waste: Effect of semi-continuous feeding on acidogenesis and methane production. Bioresour. Technol. 436, 126396 https://doi.org/10.1016/j.biortech.2021.126396.

Cheng, C.-H., Hung, C.-H., Lee, K.-S., Liau, P.-Y., Liang, C.-M., Yang, L.-H., Lin, P.-J., Lin, C.-Y., 2008. Microbial community structure of a starch-feeding fermentative hydrogen production reactor operated under different incubation conditions. Int. J. Hydrog. Energy 33, 5242–5249. https://doi.org/10.1016/j.ijhydene.2008.05.017.

Cheng, X.-Y., Liu, C., 2012. Enhanced coproduction of hydrogen and methane from cornstalks by a three-stage anaerobic fermentation process integrated with alkaline hydrolysis. Bioresour. Technol. 104, 373–379. https://doi.org/10.1016/j.biortech.2011.10.082.

Daly, S.E., Usack, J.G., Harroff, L.A., Booth, J.G., Keleman, M.P., Angenent, L.T., 2020. Systematic analysis of factors that affect food-waste storage: Toward maximizing lactate accumulation for resource recovery. ACS Sustainable Chem. Eng. 8 (37), 13934–13944. https://doi.org/10.1021/acssuschemeng.0c03161.

De Gioannis, G., Muntoni, A., Polettini, A., Pomi, R., Spiga, D., 2017. Energy recovery from one- and two-stage anaerobic digestion of food waste. Waste Manage. 68, 595–602. https://doi.org/10.1016/j.wasman.2017.06.013.

Detman, A., Bucha, M., Treu, L., Chojnacka, A., Pleśniak, Ł., Salamon, A., Łupikasza, E., Gromadka, R., Gawor, J., Gromadka, A., Drzewicki, W., Jakubiak, M., Janiga, M., Matyasik, I., Błaszczyk, M.K., Jędrysek, M.O., Campanaro, S., Sikora, A., 2021. Evaluation of acidogenesis products' effect on biogas production performed with metagenomics and isotopic approaches. Biotechnol. Biofuels 14, 1–25. https://doi.org/10.1186/S13068-021-01968-0.

Diaz-Cruces, V.F., García-Depraect, O., León-Becerril, E., 2020. Effect of lactate fermentation type on the biochemical methane potential of tequila vinasse. BioEnergy Res. 13, 571–580. https://doi.org/10.1007/s12155-020-10093-z.

Diez-Gonzalez, F., Russell, J.B., Hunter, J.B., 1995. The role of an NAD-independent lactate dehydrogenase and acetate in the utilization of lactate by *Clostridium acetobutylicum* strain P262. Arch. Microbiol. 164, 36–42. https://doi.org/10.1007/BF02568732.

EBA, 2021. EBA Statistical Report 2021, https://www.europeanbiogas.eu/eba-statistical-report-2021/ (accessed on 17 March,2022).

Favaro, L., Alibardi, L., Lavagnolo, M.C., Casella, S., Basaglia, M., 2013. Effects of inoculum and indigenous microflora on hydrogen production from the organic fraction of municipal solid waste. Int. J. Hydrog. Energy 38, 11774–11779. https://doi.org/10.1016/j.ijhydene.2013.06.137.

Feng, K., Li, H., Deng, Z., Wang, Q., Zhang, Y., Zheng, C., 2020. Effect of pre-fermentation types on the potential of methane production and energy recovery from food waste. Renew. Energy 146, 1588–1595. https://doi.org/10.1016/j.renene.2019.07.127.

Feng, K., Li, H., Zheng, C., 2018. Shifting product spectrum by pH adjustment during long-term continuous anaerobic fermentation of food waste. Bioresour. Technol. 270, 180–188. https://doi.org/10.1016/j.biortech.2018.09.035.

Forbes, H., Quested, T., O'Connor, C., 2021. Food waste index report 2021. United Nations Environment Programme. Nairobi. https://wedocs.unep.org/handle/20.500.11822/35280;jsessionid=C4B33BBDFD33D72C56C6F8F6BD1F50C1 (accessed on 17 March,2022).

FUSIONS, 2016. Estimates of European food waste levels, http://www.eu-fusions.org/phocadownload/Publications/Estimates%20of%20European%20food%20waste%20levels.pdf (accessed on 17 March 2022).

García-Depraect, O., Castro-Muñoz, R., Muñoz, R., Rene, E.R., León-Becerril, E., Valdez-Vazquez, I., Kumar, G., Reyes-Alvarado, L.C., Martínez-Mendoza, L.J., Carrillo-Reyes, J., Buitrón, G., 2021. A review on the factors influencing biohydrogen production from lactate: the key to unlocking enhanced dark fermentative processes. Bioresour. Technol. 324, 124595 https://doi.org/10.1016/j.biortech.2020.124595.

García-Depraect, O., Diaz-Cruces, V.F., León-Becerril, E., 2020. Upgrading of anaerobic digestion of tequila vinasse by using an innovative two-stage system with dominant lactate-type fermentation in acidogenesis. Fuel 280, 118606. https://doi.org/10.1016/j.fuel.2020.118606.

García-Depraect, O., Lebrero, R., Rodriguez-Vega, S., Bordel, S., Santos-Beneit, F., Martínez-Mendoza, L.J., Aragão Börner, R., Börner, T., Muñoz, R., 2022. Biodegradation of bioplastics under aerobic and anaerobic aqueous conditions: Kinetics, carbon fate and particle size effect. Bioresour. Technol. 126265 https://doi.org/10.1016/j.biortech.2021.126265.

García-Depraect, O., León-Becerril, E., 2018. Fermentative biohydrogen production from tequila vinasse via the lactate-acetate pathway: Operational performance, kinetic analysis and microbial ecology. Fuel 234, 151–160. https://doi.org/10.1016/j.fuel.2018.06.126.

García-Depraect, O., Rene, E.R., Gómez-Romero, J., López-López, A., León-Becerril, E., 2019. Enhanced biohydrogen production from the dark co-fermentation of tequila vinasse and nixtamalization wastewater: Novel insights into ecological regulation by pH. Fuel 253, 159–166. https://doi.org/10.1016/j.fuel.2019.04.147.

Guan, W., Ren, Y., Ma, X., Zhang, S., Zhao, P., Gao, M., Wang, Q., Wu, C., 2021. Preliminary determination of antibacterial substances during anaerobic preservation of food waste and their effects on methanogenesis. Environ. Technol. Innov. 24, 101813 https://doi.org/10.1016/j.eti.2021.101813.

Hung, C.-H., Chang, Y.-T., Chang, Y.-J., 2011. Roles of microorganisms other than *Clostridium* and *Enterobacter* in anaerobic fermentative biohydrogen production systems - A review. Bioresour. Technol. 102, 8437–8444. https://doi.org/10.1016/j.biortech.2011.02.084.

IEA, 2018. The role of anaerobic digestion and biogas in the circular economy. https://www.ieabioenergy.com/wp-content/uploads/2018/08/anaerobic-digestion_web_END.pdf (accessed on 17 March,2022).

Im, S., Lee, M.-K., Yun, Y.-M., Cho, S.-K., Kim, D.-H., 2020. Effect of storage time and temperature on hydrogen fermentation of food waste. Int. J. Hydrog. Energy 45, 3769–3775. https://doi.org/10.1016/J.IJHYDENE.2019.06.215.

Infantes, D., del Campo, A.G., Villaseñor, J., Fernández, F.J., 2011. Influence of pH, temperature and volatile fatty acids on hydrogen production by acidogenic fermentation. Int. J. Hydrog. Energy 36, 15595–15601. https://doi.org/10.1016/j.ijhydene.2011.09.061.

Jung, M.-Y., Mazumdar, S., Shin, S.H., Yang, K.-S., Lee, J., Oh, M.-K., 2014. Improvement of 2,3-butanodeiol yield in *Klebsiella pneumoniae* by deletion of the pyruvate formate-lyase gene. Appl. Environ. Microbiol. 80, 6195–6203. https://doi.org/10.1128/AEM.02069-14.

Kim, D.-H., Kim, S.-H., Shin, H.-S., 2009. Hydrogen fermentation of food waste without inoculum addition. Enzyme Microb. Technol. 45, 181–187. https://doi.org/10.1016/j.enzmictec.2009.06.013.

Kim, T.-H., Lee, Y., Chang, K.-H., Hwang, S.-J., 2012. Effects of initial lactic acid concentration, HRTs, and OLRs on bio-hydrogen production from lactate-type fermentation. Bioresour. Technol. 103, 136–141. https://doi.org/10.1016/j.biortech.2011.09.093.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41 https://doi.org/10.1093/nar/gks808.

Lindner, J., Zielonka, S., Oechsner, H., Lemmer, A., 2016. Is the continuous two-stage anaerobic digestion process well suited for all substrates? Bioresour. Technol. 200, 470–476. https://doi.org/10.1016/j.biortech.2015.10.052.

Matsumoto, M., Nishimura, Y., 2007. Hydrogen production by fermentation using acetic acid and lactic acid. J. Biosci. Bioeng. 103, 236–241. https://doi.org/10.1263/jbb.103.236.

Mbow, C., C. Rosenzweig, L.G. Barioni, T.G. Benton, M. Herrero, M. Krishnapillai, E. Liwenga, P. Pradhan, M.G. Rivera-Ferre, T. Sapkota, F.N. Tubiello, Y. Xu, 2019. Food Security, in: Shukla, P.R., Skea, J., Buendia, E.C., Masson-Delmotte, V., Pörtner, H.-O., Roberts, D.C., Zhai, P., Slade, R., Connors, S., van Diemen, R., Ferrat, M., Haughey, E., Luz, S., Neogi, S., Pathak, M., Petzold, J., Pereira, J.P., Vyas, P., Huntley, E., Kissick, K., Belkacemi, M., Malley, J. (Eds.), Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. Summary for Policymakers. In press.

Moon, C., Jang, S., Yun, Y.-M., Lee, M.-K., Kim, D.-H., Kang, W.-S., Kwak, S.-S., Kim, M.-S., 2015. Effect of the accuracy of pH control on hydrogen fermentation. Bioresour. Technol. 179, 595–601. https://doi.org/10.1016/j.biortech.2014.10.128.

Pipyn, P., Verstraete, W., 1981. Lactate and ethanol as intermediates in two-phase anaerobic digestion. Biotechnol. Bioeng. 23, 1145–1154. https://doi.org/10.1002/bit.260230521.

Peinemann, J.C., Demichelis, F., Fiore, S., Pleissner, D., 2019. Techno-economic assessment of non-sterile batch and continuous production of lactic acid from food waste. Bioresour. Technol. 289, 121631 https://doi.org/10.1016/j.biortech.2019.121631.

Sarkar, O., Rova, U., Christakopoulos, P., Matsakas, L., 2021. Influence of initial uncontrolled pH on acidogenic fermentation of brewery spent grains to biohydrogen and volatile fatty acids production: Optimization and scale-up. Bioresour. Technol. 319, 124233 https://doi.org/10.1016/j.biortech.2020.124233.

Shen, F., Yuan, H., Pang, Y., Chen, S., Zhu, B., Zou, D., Liu, Y., Ma, J., Yu, L., Li, X., 2013. Performances of anaerobic co-digestion of fruit & vegetable waste (FVW) and food waste (FW): Single-phase vs. two-phase. Bioresour. Technol. 144, 80–85. https://doi.org/10.1016/j.biortech.2013.06.099.

Tang, J., Wang, X.C., Hu, Y., Zhang, Y., Li, Y., 2017. Effect of pH on lactic acid production from acidogenic fermentation of food waste with different types of inocula. Bioresour. Technol. 224, 544–552. https://doi.org/10.1016/j.biortech.2016.11.111.

Ventura, M., Margolles, A., Turroni, F., Zomer, A., de los Reyes-Gavilán, C., van Sinderen, D., 2011. Stress responses of Bifidobacteria. In: Tsakalidou, E., Papadimitriou, K. (Eds.), Stress Responses of Lactic Acid Bacteria. Springer, Boston, pp. 305–322.

Weiss, S., Xu, Z.Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J.R., Vázquez-Baeza, Y., Birmingham, A., Hyde, E.R., Knight, R., 2017. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome. 5 https://doi.org/10.1186/s40168-017-0237-y.

Wu, S., Xu, S., Chen, X., Sun, H., Hu, M., Bai, Z., Zhuang, G., Zhuang, X., 2018. Bacterial communities changes during food waste spoilage. Sci. Rep. 8, 1–9. https://doi.org/10.1038/s41598-018-26494-2.

Wu, Y., Ma, H., Zheng, M., Wang, K., 2015. Lactic acid production from acidogenic fermentation of fruit and vegetable wastes. Bioresour. Technol. 191, 53–58. https://doi.org/10.1016/j.biortech.2015.04.100.

Wu, Y., Wang, C., Liu, X., Ma, H., Wu, J., Zuo, J., Wang, K., 2016. A new method of two-phase anaerobic digestion for fruit and vegetable waste treatment. Bioresour. Technol. 211, 16–23. https://doi.org/10.1016/j.biortech.2016.03.050.

Yang, L., Chen, L., Li, H., Deng, Z., Liu, J., 2022. Lactic acid production from mesophilic and thermophilic fermentation of food waste at different pH. J. Environ. Manage. 304, 114312 https://doi.org/10.1016/j.jenvman.2021.114312.

Zhang, Z., Tsapekos, P., Alvarado-Morales, M., Angelidaki, I., 2021. Bio-augmentation to improve lactic acid production from source-sorted organic household waste. J. Clean. Prod. 279, 123714 https://doi.org/10.1016/j.jclepro.2020.123714.

Zhou, M., Yan, B., Wong, J.W.C., Zhang, Y., 2018. Enhanced volatile fatty acids production from anaerobic fermentation of food waste: A mini-review focusing on acidogenic metabolic pathways. Bioresour. Technol. 248, 68–78. https://doi.org/10.1016/j.biortech.2017.06.121.