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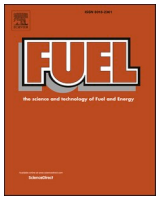

Full Length Article

Elucidating the role of pH and total solids content in the co-production of biohydrogen and carboxylic acids from food waste via lactate-driven dark fermentation

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ABSTRACT

Notwithstanding lactate-driven dark fermentation (LD-DF) can cope with inhibition issues associated with the over-proliferation of lactate producers, there is still a knowledge gap about the role of key operational parameters. In this study, the effect of pH and total solids (TS) content on the co-production of hydrogen and carboxylic acids, including medium-chain carboxylic acids (MCCAs), from food waste (FW) via LD-DF was investigated. A series of batch fermentations was conducted, first, without pH control, and then at fixed pH values of 5.5, 6.0 and 6.5, while maintaining constant the TS content at 5 %. It was observed that the higher the operational pH, the lower the accumulation of lactate and the higher the extent and rate of hydrogen production, sustaining a maximum hydrogen production yield and rate of 81 NmL/g VS fed and 9 NL/L-d, respectively, at pH 6.5. In a second series of batch tests, the TS content was adjusted to 5, 7.5 and 10 % while pH was set at 6.5. The highest hydrogen production performance (103 NmL/g-VS fed and 13.3 NL/L-d) was achieved at 7.5 % TS, which also resulted in the highest accumulation of MCCAs, particularly of caproate, with an associated titer of 8.7 g/L. Hydrogen production plateaued with the exhaustion of lactate regardless of the condition tested. Further assessment through biochemical methane potential tests showed that LD-DF effluents can be alternatively valorised into biogas. Overall, the results obtained confirmed the key role of pH and TS content in the LD-DF of FW and suggested that this non-conventional route may be an alternative approach to cope with lactate flux diverted toward undesirable non-hydrogen-producing metabolic pathways.

1. Introduction

The exponential production and accumulation of food waste (FW) has become one of the main challenges to overcome worldwide [1]. FW can be defined as all edible and inedible parts of food that are removed from the food supply chain, excluding food used for conversion/valorization purposes through bio-based processes or other industrial uses (e.g., animal feed) [2]. The Food and Agriculture Organization (FAO) estimates that nearly a third part of the food produced globally ends up becoming FW along the food consuming chain causing not only economic losses but also social and environmental damage [1]. Most FW ends up on landfills and ultimately generates gaseous (CH_4, CO_2, NH_3) and liquid (leachate) emissions. Other management options like composting, anaerobic digestion and thermal conversion represent a much lower percentage of the FW treatments worldwide, mostly due to a reduced scalable capacity, even though FW contains high energy value [3,4]. In Europe, approx. 88 Mt of FW are produced annually, with an associated emission of 186 Mt/year of carbon dioxide (CO_2), 1.7 Mt/year of sulphur dioxide and 0.7 Mt/year of phosphates, being equivalent to 15 % of all environmental damage caused by the food supply chain in Europe [2].

FW is a suitable feedstock for the production of different high-value products via fermentation owing to its high energy content and carbon density [5–7]. Among these bioproducts, hydrogen has gained a tremendous attention as a clean energy substitute of fossil-based fuels because of its high energy density (2.7-fold higher than other hydrocarbon fuels) combined with the fact that it does not generate CO_2 when is combusted (only water vapor) [8,9]. There is a diverse group of

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biotechnologies able to produce hydrogen (e.g., dark fermentation (DF), photofermentation, biophotolysis, microbial electrolysis cells). Among them, the DF process has shown relatively superior hydrogen production yields and rates, and flexibility, to manage different types of organic wastes and wastewaters [10]. Furthermore, DF allows for the valorization of FW not only through the production of renewable hydrogen but also of carboxylic acids that can be further used to produce other high added value products, such as biopolymers (polyhydroxyalkanoates) [3]. In this context, the production of medium-chain carboxylic acids (MCCAs) opens new routes for FW valorization into green chemicals. Indeed, caproate or heptanoate can be employed for the production of antimicrobial agents, food additives, pharmaceuticals, fragrances, as well as diesel and aviation fuels [11]. The acidogenic effluent of DF, rich in organic acids, can be also fermented by methanogenic bacteria to produce biogas [11,12].

However, despite the numerous efforts made in the DF field over the last two decades, the DF of FW is still challenging, deserving more research to make the process more stable and efficient in terms of hydrogen yield and productivity. Evidence in the literature has shown that the impairment in hydrogen efficiency is mostly caused by the overgrowth of lactic acid bacteria (LAB), which are naturally and ubiquitously present in FW and therefore can thrive and proliferate during its storage, transport and disposal chain [10]. The negative effect of LAB in the DF process has been ascribed to the competition with hydrogen-producing bacteria (HPB) for substrate, the acidification of the culture broth due to the production and accumulation of lactate, and the excretion of antimicrobial/inhibitory compounds to the culture broth [10]. In this context, sterilization of the substrate has been proposed to prevent LAB proliferation during DF, making the process more expensive and complex, although this strategy has shown little success since LAB end up thriving again under long-term operation [10,13]. Recently, it has been proposed to tailor the DF towards lactate-utilizing, hydrogen-producing pathways to cope with the presence of LAB [10]. The lactate-driven DF (LD-DF) approach differs from its conventional DF counterpart on the fact that lactate (derived from carbohydrates) is used to produce hydrogen directly. Contrarily, carbohydrates are the hydrogen precursor in the common DF process. It should be noted that the role of lactate in the DF process is commonly overlooked. The LD-DF would benefit from LAB through a cooperative association with lactate-utilizing HPB [14–16]. Thus, the major advantage of the LD-DF process relies on the fact that lactate produced by lactic bacteria is harnessed to produce hydrogen, otherwise it would be accumulated in the fermentation broth and in turn result in low hydrogen productions. Several recent studies have endorsed the importance of the LD-DF process to increase the recovery of hydrogen from a number of feedstocks [17–23]. Besides making the process more cost-effective and less complex since it enables to avoid pretreatments aimed to kill LAB, the LD-DF may bring about some desirable bioactivities such as pH regulation (due to the production and consumption of lactate), substrate hydrolysis, biomass retention, oxygen depletion and substrate detoxification [10]. Despite these latent process advantages, studies are lacking to support LD-DF as a viable option to produce hydrogen from FW. Furthermore, although the impact of some key process parameters such as pH and total solids (TS) content on the DF process performance have been extensively studied [24], there is still limited knowledge about their effect on the LD-DF process of FW [17]. This study aimed at evaluating the production of renewable hydrogen with the concomitant formation of organic acids, including MCCAs, from FW through the LD-DF process. Emphasis was paid on investigating the effect of operational pH and TS content on the hydrogen production yield and the maximum volumetric hydrogen production rate, and on the titer and distribution of organic acids involved. The methanogenic potential of the acidogenic effluent obtained at best observed operational conditions was further evaluated using conventional biochemical methane potential (BMP) tests.

2. Materials and methods

2.1. Inocula and feedstock

The acidogenic inoculum source was digestate obtained from a pilot-scale anaerobic digester treating FW under mesophilic conditions. Heat-shock pretreatment (90 °C for 20 min) was used to kill methanogens. Three cycles of subculturing were carried out using the pre-treated microbial culture as the inoculum and lactose as the carbon source, according to García-Depraect et al. (2022) [25]. The resulting enriched mixed culture was thus used as the inoculum, which has been previously characterised to be composed of the genera *Lactobacillus* (55 %), *Klebsiella* (28 %), *Clostridium* (11 %), *Stenotrophomonas* (3 %), *Acinetobacter* (1.8 %), among others [25]. Similarly, a fresh inoculum for the BMP tests was obtained from the mesophilic anaerobic digester of the Valladolid (Spain) wastewater treatment plant. The methanogenic inoculum was preincubated at 37 °C under anaerobic conditions for 7 days before inoculation. The preincubated anaerobic sludge exhibited a pH of 7.5 and a TS and volatile solids (VS) content of 15.8 and 8.63 g/L, respectively.

Simulated FW was used as a model substrate following the recipe reported by Neves et al. (2008) [26] to mimic restaurant FW. This FW was based on a grinded mixture of potato flakes (78 %), chicken breast (14 %), white cabbage (4 %) and pork lard (4 % *w/w*), as a source of carbohydrates, proteins, and lipids, respectively. A 17.5-kg batch of FW was prepared and stored in several plastic bags at −20 °C until use. The pH of the FW was 6.2 ± 0.05, while its COD and TS concentration accounted for 295 g O_2/kg and 211 g TS/kg, respectively. The composition of the FW (% *w/w* on a dry basis) was as follows: carbohydrates (50.7 ± 3.8), proteins (25.4 ± 0.3), lipids (20.03) and ash (4.7). Such proportions were well associated with an ultimate analysis based on carbon (C; 50.3 ± 0.7 %), hydrogen (H, 7.3 ± 0.3 %), oxygen (O, 33.6 ± 0.2 %), nitrogen (N, 4.1 ± 0.1 %) and phosphorus (P, 0.3 %). No sulphur (S) was detected.

2.2. Experimental set-up and evaluation of operational conditions for hydrogen production

Batch experiments were performed in two identical 1-L glass stirred tank reactors with a working volume of 0.8 L. The LD-DF of FW was carried out at 37 °C under magnetic stirring at ≈ 200 rpm. The fermentations were conducted with a cultivation broth initially composed of 720 mL synthetic FW (previously grinded for 3 min using a conventional kitchen blender), and inoculum at 10 % (*v/v*) with a volatile suspended solids (VSS) concentration of 0.32 ± 0.03 g/L. The hydrogenogenic inoculum was preincubated overnight at 37 °C using lactose as the sole carbon source at a concentration of 10 g/L in 2.1 L gas-tight glass flasks with 0.9 L of mineral salt medium (the pre-inoculum size was 10 % *v/v*). The composition of the growth medium was (in g/L) as follows: NH_4Cl, 2.4; K_2HPO_4, 2.4; $MgCl_2$, 1.18; KH_2PO_4, 0.6; $CaCl_2$, 0.11; and $FeCl_2$, 0.024.

In a first series of batch tests, the TS content was fixed at 5 % and the influence of pH on the LD-DF of FW was investigated by automatically controlling it at 5.5, 6.0, and 6.5. Additionally, a fermentation was also performed without pH control (initial pH of 5.9). In a second series of batch tests, the influence of TS concentration was investigated by adjusting the TS content at 5, 7.5 and 10 %, while maintaining constant the best operational pH previously determined (i.e., 6.5). TS content was adjusted using tap water. Both the operational pH values and the initial TS contents were chosen based on previous results [17]. All experimental conditions were conducted in duplicate. The pH of the fermentation was automatically controlled by adding NaOH 3 M or HCl 3 M with a pH controller (BSV, EVOPH-P-5 model, Spain). Gas production and composition, pH, organic acids, and the cumulative volume of NaOH/HCl were periodically measured. The performance of the process was measured based on the rates and yields of hydrogen production, VS

removal efficiency and organic acids profile.

2.3. Biochemical methane potential tests

The BMP of the acidogenic broth collected from the fermentations performed at the best pH (i.e., 6.5) and TS content (i.e., 7.5 %) was evaluated in order to elucidate the potential enhancement in the methane yield and kinetics supported by the LD-DF process. The substrates tested were the acidogenic broths collected after 24 and 48 h of fermentation time. These sampling points were chosen based on the cultivation times at which the production of hydrogen (24 h) and organic acids (48 h) peaked. Additionally, non-fermented FW was also evaluated to simulate a one-stage anaerobic digestion process. The BMP assays were performed in 2.1 L gas-tight glass flasks with 0.5 L of working volume, at a constant temperature (37 °C) and agitation rate (4.5 rpm) using a roller shaker (Wheaton Scientific Products, USA). The BMP tests were conducted at a fixed substrate to inoculum ratio of 0.25 (on a VS basis), supplemented with 5 g/L of $NaHCO_3$ as buffering agent to prevent acidification of the medium, and flushed with helium for 5 min to remove residual oxygen from the headspace. Blanks containing only inoculum with 5 g/L $NaHCO_3$ were set to quantify the endogenous biogas production, while microcrystalline cellulose (Merck ltd., Germany, CAS number 9004–34–6) was used as the positive control, as recommended by Holliger et al. (2016) [27]. All BMP tests were carried out in triplicate. BMP was estimated using the manometric method, recording and releasing the overpressure in the headspace to achieve atmospheric pressure before each biogas measurement. The headspace pressure and the composition (CO_2 and CH_4) of biogas were periodically measured, avoiding overpressure on the gas-tight reactors (>200 mbars). The final pH of the broth and organic acids concentrations were also measured at the end of the experiment.

2.4. Analytical methods

Gas composition (i.e., H_2, CO_2 and CH_4) was measured by gas chromatography using a Varian CP-3800 gas chromatograph (GC) coupled with a thermal conductivity detector (TCD) and equipped with 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), using helium as the carrier gas, according to Alcántara et al. (2015) [28]. Organic acids (i.e., lactic acid, acetic acid, formic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, caproic acid, isocaproic acid, and heptanoic acid) were measured by high-performance liquid chromatography (HPLC) using a Alliance HPLC system (model e2695, USA) equipped with an ultraviolet (UV) detector (214 nm) and an Aminex chromatographic column kept at 75 °C (HPX-87H, Bio Rad, USA), preceded by a Micro-Guard Cation H + refill cartridge of 30 × 4.6 mm (Bio Rad, USA) as a pre-column. Sulfuric acid 25 mM was used as the eluent at a flow rate of 0.7 mL/min. Due to their expected low titers at the end of the BMP assays, organic acids were quantified by gas chromatography using a Agilent GC (7820A, Agilent, USA) equipped with a flame ionization detector (FID) and a packed column (10 % SP-1000 + 1 %H_3PO_4 on Chromosorb® W acid washed 100/120 mesh size, 2 m × 3.175 mm; Teknokroma, Spain) [29]. The temperatures of the injection port and detector were kept both at 350 °C. The oven temperature was initially maintained at 135 °C for 10 min, then it was increased to 151 °C at a rate of 3 °C/min, and finally ramped at 8 °C/min to 180 °C and held for 5 min. Nitrogen, at a flow rate of 45 mL/min, was employed as the carrier gas. The flow rate of hydrogen and air was 45 and 350 mL/min, respectively [25]. Carbohydrates were measured using the phenol–sulphuric acid method based on the digestion of 1 mL of sample with 0.6 mL of phenol at 5 % *v/v* and 3.6 mL of sulphuric acid at 95 % *v/v*. Concentration was determined by the absorbance method using an Spectrophotometer Star Nano from BMG LACTECH. Protein content was estimated using the total nitrogen concentration measured by the Kjeldahl method [30], with a nitrogen-to-protein index of 6.25 [25]. Lipid content was analysed using the gravimetric method performed by the Regional Service for Agri-food Research and Development (SERIDA, Spain). The elemental composition of FW (C, H, O, N and S) was analysed using an elemental analyzer EA FLASH 2000 (Thermo Fisher Scientific) coupled with a TCD detector and a Mettler Toledo XP6 microscale, using helium as a carrier (140 mL/min) and reference (100 mL/min) gas coupled with oxygen (250 mL/min) at 900 °C furnace temperature for C, H, N, and S measurements; and helium as carrier (130 mL/min) and reference (100 mL/min) gas at 1060 °C furnace temperature for O measurement; based on the internal procedure of the Central Instrumental Laboratories of the University of Burgos (Spain). Finally, P content was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) based on the internal procedure of the Laboratory of Instrumental Techniques at the University of Valladolid (Spain).

2.5. Data treatment

Data on the cumulative hydrogen and methane production were further processed using the modified Gompertz kinetic model, according to Eq. (1), where *Hmax* is the maximum cumulative hydrogen or methane production (NmL), *Rmax* is the maximum production rate of hydrogen or methane (NmL/h and NmL/d for hydrogen and methane, respectively), λ is the duration of *lag* phase (t), and *t* is the incubation time (in hours and days for hydrogen and methane production, respectively).

$$H = Hmax^{*}\exp\left\{ -exp\left[\frac{Rmax^{*}e}{Hmax}^{*}(\lambda - t) + 1\right]\right\} \quad (1)$$

The methane yield from the BMP assays was analysed according to Eq. (2) [31], where *BMP* is the volume of methane produced per gram of VS added (NmL CH_4/g VS added), V_S is the mean value of the accumulated volume of methane produced from the substrate (NmL), V_B is the mean value of the accumulated volume of methane derived from the control blank (NmL), m_{IS} and m_{IB} stands for the total amount of inoculum (g VS) in the substrate and blank, respectively, and m_{VS} is the total amount of substrate (g VS).

$$\mathrm{BMP} = \left[V_S\text{-}\left(\mathrm{VB}\frac{\mathrm{mIS}}{\mathrm{mIB}}\right)\right]/\mathrm{m}_{VS} \quad (2)$$

The theoretical maximum methane production of the model substrate was calculated based on Eq. (3) [32], where: Y_c, Y_p and Y_l are the methane yields of carbohydrates (0.395 NmL CH_4/g), proteins (0.5 NmL CH_4/g) and lipids (0.854 NmL CH_4/g), respectively. W_c, W_p, and W_l stand for the empirical content of carbohydrate, proteins and lipids in the model substrate, respectively [32].

$$Y\left(\frac{m^3CH_4}{Kg}\right) = Y_c{}^{*}W_c + Y_p{}^{*}W_p + Y_l{}^{*}W_l \quad (3)$$

The acetate produced by homoacetogenesis ($Ac_{homoacet}$) was estimated based on Eq. (4) [33], where [Ac], [But], [Prop] and [H_2] are the concentrations (in mmol) of acetate, butyrate, propionate and hydrogen, respectively.

$$Ac_{homoacet} = (2[Ac] + 2[But] - [Prop] - [H_2])/6 \quad (4)$$

Bioconversion ratios (BR) were calculated as a measure of the degree of acidification, according to Eq. (5), where COD eq and C eq is the sum of COD equivalent and carbon concentrations (in g/L), respectively, of all the organic acids measured at the end of the process, and $TCOD_{FW}$ and TC_{FW} is the total COD and carbon content (in g /L) of the FW fed, respectively. The COD equivalence of organic acids was determined according to Eqs. (6) and (7), where OA (organic acid) is the molecular formula of a given organic acid, and *a*, *b*, *c*, and *d* stand for the number of moles of OA, O_2, H_2O, and CO_2, respectively, based on the analysis of stoichiometry for complete combustion.

$$BR(\%) = \frac{\text{COD eq (or C eq)}}{TCOD_{FW}(orTC_{FW})} x100 \quad (5)$$

$$aOA + bO_2 \rightarrow cH_2O + dCO_2 \quad (6)$$

$$COD_{equiv.} = \frac{aO_2}{bOA} \quad (7)$$

3. Results and discussion

3.1. Influence of pH and TS concentration on the lactate-based fermentative hydrogen production

At a fixed TS of 5 %, the highest hydrogen yield (80.9 NmL/g VS fed) was achieved at a pH of 6.5, which was 46 and 9.6 % higher than that recorded at a constant pH of 5.5 and 6.0, respectively (Table 1). As expected, it was found that the modified Gompertz kinetic model (Eq. (1)) adequately described the cumulative hydrogen production experimentally recorded, with coefficients of determination above 0.99 regardless of the pH and TS concentration tested (Fig. 1, Table 1). Based on the estimated kinetic parameters, the maximum hydrogen production potential (*Hmax*) achieved in the assays conducted at a fixed TS concentration of 5 % and a pH of 5.5, 6.0 and 6.5 accounted for 2.7, 3.6 and 3.9 NL/L of reactor, respectively, with corresponding maximum volumetric hydrogen production rates (*Rmax*) of 67.4, 204.4 and 373.9 NmL/L-h. In contrast, no hydrogen was produced in the assay conducted without pH control due to the rapid acidification of the cultivation broth, which reached a pH < 5 in less than 2 h of fermentation. The VS removal efficiencies recorded at the end of the fermentations were of 48.0, 53.8 and 52.7 % for pH 5.5, 6.0 and 6.5, respectively. No methanogenic activity was observed during the test period for all experimental conditions tested. This test series confirmed the key role of pH in governing the efficiency of the LD-DF of FW in terms of hydrogen production yield and rate. The results obtained confirmed a clear improvement in hydrogen production performance when approaching neutral pH values.

The operational pH is one of the key operational factors in the DF process because it greatly affects not only the microbial structure but also the metabolism in relation to the metabolic fluxes, biocatalytic activity (including hydrogen-producing enzymes), biomass growth and substrate degradation [10,24,34]. Previous studies have showed that hydrogen production via LD-DF is heavily impacted by pH, occurring at pH values between 3.8 and 7.5, but mostly in the pH range of 5–7, and obtaining superior performances at pH values of 5.5–6.5 [10]. It has been argued that a suitable balance between the production of lactate by LAB and its further consumption by HPB is required in the LD-DF, and that pH affects such microbial equilibrium [10,15]. It was thus hypothesized that a fixed pH of 5.5 somehow affected the balance between HPB and LAB, been more conducive to the growth of LAB as endorsed by the higher accumulation of lactate recorded (see section 3.2). Contrarily, a near-neutral pH might ensure balance and syntrophy between LAB and lactate-utilizing HPB, allowing them to co-exist, thus leading to lower lactate accumulation and higher hydrogen production. Further molecular analyses are obviously required to prove this hypothesis. Nonetheless, it seems a plausible assumption that can explain the clear trends

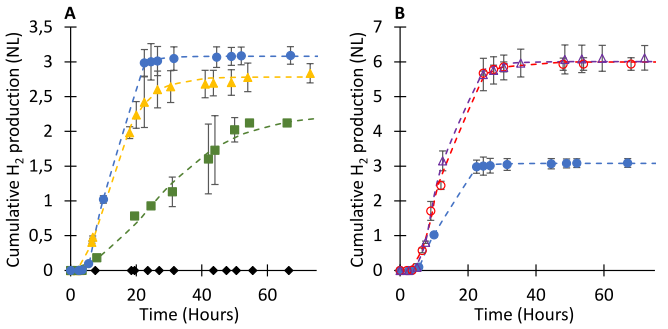

Fig. 1. Time course of the accumulated hydrogen (H_2) produced at different pH values (A) and TS contents (B). The pH tested were 5.5 (■), 6.0 (▲), 6.5 (●), and no pH control (◆). The TS contents tested were 5% (●), 7.5% (○), and 10% (△).

observed: the higher the culture pH, the lower the accumulation of lactate and the higher the extent and rate of hydrogen production.

The best pH found for hydrogen production of 6.5 was thus set as operational pH in the test series assessing the influence of TS content on the process. It was found that a TS content of 7.5 % supported the highest hydrogen yield (103.4 NmL/g VS fed), which was ≈ 29 % higher than that exhibited at 5 and 10 % TS concentrations. The maximum cumulative hydrogen production recorded at 5, 7.5 and 10 % TS was 3.8, 7.4 and 7.6 NL/L of reactor, respectively, with corresponding *Rmax* values of 373.9, 556.1 and 603.7 NmL/L-h, respectively (Table 1), and associated VS removal efficiencies of 52.5, 55.4 and 59.8 %, respectively. The increase in hydrogen productivities observed when the TS concentration (which is positively correlated with substrate concentration) was increased from 5 to 7.5 % could be explained by the preference of the microbiota towards lactate-utilizing, hydrogen-producing pathways at higher FW concentration. Wu et al. (2012) [35] observed that a higher substrate concentration could enhance the hydrogen production using lactate and acetate as substrates because electron equivalents required for biomass growth are rapidly satisfied, leaving an excess of electron equivalents for hydrogen production. However, it should be noted that the apparent viscosity of the fermentation broth at 10 % TS was too high and led to an inefficient magnetic stirring, which in turn may not only impaired the contact between substrate and biocatalyst but also the hydrogen mass transfer from the liquid to gas phase, and therefore might have negatively affected the FW-to-hydrogen bioconversion. Previous studies have reported a severe decrease in hydrogen yield at too high TS concentrations, especially when surpassing the 15 % TS content (dry DF) due to an increase of lactate production in opposition to hydrogen production [36,37]. It is well known that a suitable balance between hydrolytic and fermentative bioactivities is a prerequisite for efficient hydrogen production, especially using complex particulate substrates [38,39]. In this context, it has been previously hypothesized that the presence of particulate material may alter the balance between LAB and HPB in the LD-DF, leading to higher LAB activities at higher TS content, while lower TS contents may boost the activity of HPB [40]. Although the maximum TS content herein tested remained below 15 %, the accumulation of lactate at 10 % TS was

Table 1
Hydrogen production yields and Modified Gompertz model kinetic data obtained in the assays conducted at different pH and TS content.

Condition		H_2/L (NmL)	Yield (NmL H_2/g VS fed)	Yield (NmL H_2/g CH fed)	*P* (NmL)	*Rmax* (NmL/h)	λ (h)	R^2
pH	No pH control	0	0	0	0	–	–	–
	5.5	2651	55.4	116.0	2263	54.0	7.4	0.9954
	6.0	3546	74.1	155.2	2782	163.5	4.5	0.9990
	6.5	3869	80.9	169.4	3080	299.2	6.6	1.0000
TS (%)	7.5	7423	103.4	216.4	5922	444.9	6.3	0.9996
	10 %	7643	79.9	166.9	6005	483	6.1	0.9997

CH: carbohydrates.

comparatively higher than that at 5 and 7.5 % (as will be discussed in section 3.2), which might also explain the decrease in hydrogen yield when augmenting the TS from 7.5 to 10 %.

The extent of *lag* phase is other parameter highly influenced by the culture pH. The shortest *lag* phase (based on the modified Gompertz model parameter λ) of 4.5 h was recorded at pH 6.0, followed by the 6.3 h determined at a pH of 6.5 regardless of the TS content. A longer *lag* phase, on average, of 7.3 h was observed at a pH of 5.5. Thus, the length of *lag* phase at pH 6.0 was 38.5 and 62 % shorter than those at pH 6.5 and 5.5, respectively. It is worth highlighting that the higher *lag* phase recorded for pH 5.5 reinforces the hypothesis based on the balanced growth between LAB and HPB outlined above. In this context, Dareioti et al. (2014) [41] observed shorter *lag* phases at increasing pH values during the LD-DF of an organic mix containing (in volume) 55 % olive

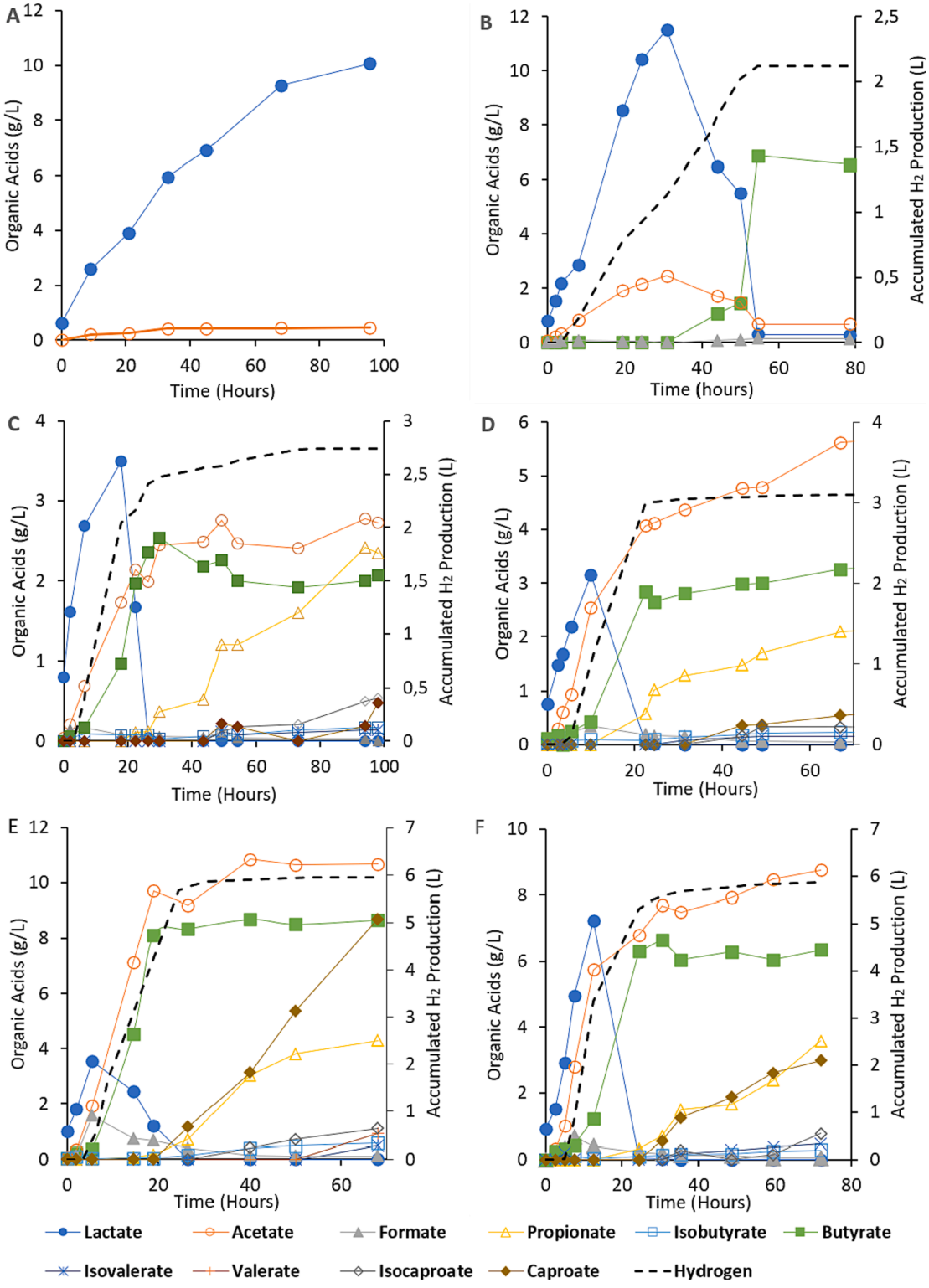

Fig. 2. Time course of organic acids concentration and hydrogen (H_2) production at A) 5% TS and no pH control; B) 5% TS and pH 5.5; C) 5% TS and pH 6.0; D) 5% TS and pH 6.5; E) 7.5% TS and pH 6.5, and F) 10% TS and pH 6.5.

mill wastewater, 40 % cheese whey and 5 % liquid cow manure by a mixed acidogenic culture under mesophilic conditions. In that study, the shortest *lag* phase of 26.1 h was recorded at a pH of 6.5, which was 3.5 times shorter than that at pH 5.5. Similarly, García-Depraect et al. (2019a) [15] evaluated the influence of pH on the mesophilic LD-DF of tequila vinasses by a mixed culture and found that the duration of the *lag* phase was shortened from 111.6 h at a constant pH of 5.5 to 28.5 h at a pH of 6.5. Indeed, the duration of the hydrogen production phase increased at decreasing pH values. The end of the hydrogen production phase in this study was estimated as the process time where 95 % of the total hydrogen production was reached (t_{95}). Hence, the shortest t_{95} occurred at a pH of 6.5 (23.9 ± 2.1 h) regardless of the TS content tested. Such a t_{95} was 20.8 and 161.6 % shorter than the assays performed at pH 6.0 and pH 5.5, respectively.

The hydrogen yields and rates available in the literature vary significantly depending on several factors such as the type of operational conditions, feedstock and inoculum used (see Supplementary material). Thus, the observed yields in the mesophilic DF of FW ranged from 50 to 200 NmL H_2/g VS fed, while the reported hydrogen productivities or maximum volumetric hydrogen production rates rarely exceed 1 NL/L-d [42–46]. For instance, Danko et al. (2008) [47] recorded 154.8 NmL H_2/g VS fed and 100 NmL H_2/L-h at a pH of 5.5 using a simulated FW with a formulation similar than that used in the present study. Similarly, Moreno-Andrade et al. (2015) [48] reported a maximum hydrogen productivity of 334.0 ± 56.4 NmL/L-h during the semi-continuous DF of cafeteria FW by a heat shock-pretreated mixed culture at a hydraulic retention time (HRT) of 24 h and 35 °C. Here it is worth noting that the fraction of carbohydrates present in the substrate represents one of the key factors influencing hydrogen production [45,49,50]. For instance, Alibardi and Cossu (2016) [45] observed a clear correlation between hydrogen production and the percentage of carbohydrates in different FW mixtures, with yields ranging from 189.7 to 229.4 NmL H_2/g carbohydrate fed at a pH of 5.5, which are similar to the 116 and 216 NmL H_2/g carbohydrate recorded in this work (Table 1). It should be noted that hydrogen is mainly produced from lactate rather than from carbohydrates in the LD-DF process, however, carbohydrates are still needed to produce lactate [10]. Overall, the process performance indicators, mainly the outstanding hydrogen productivities (up to ~ 600 NmL/L-h) herein recorded, confirmed the potential advantage of the LD-DF process for FW bioconversion to biohydrogen.

3.2. pH and TS effect on organic acids production

Different organic acids profiles were recorded in the fermentation broth depending on the pH and TS content tested (Fig. 2). In the absence of pH control, neither hydrogen nor CO_2 were produced, but a gradual conversion of the substrate into lactate of up to 10.1 ± 1.1 g/L was observed by the end of the process. The production of lactate slowed down throughout the fermentation process, although it continued despite the extremely acid conditions (pH < 4) prevailing during the fermentation. The average acidification degrees (BR) obtained based on the total content of COD in the FW and the corresponding equivalent concentrations of measured organic acids were between 16.0 and 56.1 %, which were comparable to those estimated on a carbon basis regardless of the condition tested (Table 2). The test performed at pH 6.5 and 7.5 % TS exhibited the highest BR equivalent to a yield of 0.8 g COD/g VS added, which is comparable with yields previously reported for FW (see Supplementary material). The lowest acidification degree

Table 2
Comparison of the COD and carbon equivalents for the different organic acids recorded at the end of the process and associated bioconversion ratio (BR, acidification degree) for the different pH and TS contents evaluated.

Organic acid	COD eq (g COD/L)	BR (% COD)	C eq (g C/g L)	BR (% C)	COD eq (g COD/g L)	BR (% COD)	C eq (g C/g L)	BR (% C)
	No pH Control (5 % TS)				**pH 5.5 (5 % TS)**			
Lactate	10.7 ± 1.2	15.3 ± 1.7	4.0 ± 0.4	16.1 ± 1.7	0.3 ± 0.4	0.4 ± 0.6	0.1 ± 0.2	0.4 ± 0.6
Acetate	0.5 ± 0.1	0.7 ± 0.1	0.2 ± 0.03	0.7 ± 0.1	0.7 ± 0.6	1.0 ± 0.9	0.3 ± 0.2	1.1 ± 1.0
Formate	0.0	0.0	0.0	0.0	0.0	0.1 ± 0.04	0.0	0.1 ± 0.1
Propionate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Isobutyrate	0.0	0.0	0.0	0.0	0.0	0.1 ± 0.1	0.0	0.1 ± 0.1
Butyrate	0.0	0.0	0.0	0.0	12.2 ± 0.4	17.4 ± 0.6	3.7 ± 0.1	14.6 ± 0.5
Isovalerate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Valerate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Isocaproate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Caproate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	11.2 ± 1.3	16.0 ± 1.8	4.2 ± 0.5	16.8 ± 1.9	13.3 ± 1.6	19.0 ± 2.3	4.1 ± 0.6	16.4 ± 2.3
	pH 6.0 (5 % TS)				**pH 6.5 (5 % TS)**			
Lactate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acetate	3.0 ± 0.5	4.2 ± 0.7	1.1 ± 0.2	4.4 ± 0.8	6.0 ± 1.0	8.6 ± 1.4	2.3 ± 0.4	9.0 ± 1.5
Formate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Propionate	3.6 ± 0.5	5.2 ± 0.8	1.2 ± 0.2	4.7 ± 0.7	3.2 ± 1.6	4.5 ± 2.3	1.0 ± 0.5	4.1 ± 2.1
Isobutyrate	0.3 ± 0.1	0.4 ± 0.2	0.1 ± 0.03	0.4 ± 0.2	0.4 ± 0.1	0.6 ± 0.1	0.1 ± 0.03	0.5 ± 0.1
Butyrate	3.6 ± 2.1	5.2 ± 3.0	1.1 ± 0.6	4.3 ± 2.5	5.9 ± 0.02	8.4 ± 0.03	1.8 ± 0	7.1 ± 0.03
Isovalerate	0.3 ± 0.4	0.4 ± 0.6	0.1 ± 0.1	0.3 ± 0.4	0.3 ± 0.4	0.4 ± 0.6	0.1 ± 0.1	0.4 ± 0.5
Valerate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Isocaproate	1.1 ± 1.5	1.6 ± 2.2	0.3 ± 0.4	1.2 ± 1.7	0.7 ± 1.0	1.0 ± 1.5	0.2 ± 0.3	0.8 ± 1.1
Caproate	0.4 ± 0.6	0.6 ± 0.8	0.1 ± 0.2	0.5 ± 0.7	1.2 ± 1.7	1.7 ± 2.4	0.3 ± 0.5	1.4 ± 1.9
Total	12.3 ± 5.8	17.6 ± 8.3	4.0 ± 1.8	15.9 ± 7.1	17.7 ± 5.9	25.4 ± 8.4	5.8 ± 1.8	23.2 ± 7.3
	7.5 % TS (pH 6.5)				**10 % TS (pH 6.5)**			
Lactate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acetate	11.4 ± 1.5	10.8 ± 1.4	4.3 ± 0.6	11.4 ± 1.5	9.3 ± 1.5	6.7 ± 1.1	3.5 ± 0.6	7.0 ± 1.1
Formate	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Propionate	6.5 ± 0.7	6.2 ± 0.7	2.1 ± 0.2	5.6 ± 0.6	5.4 ± 1.5	3.9 ± 1.1	1.7 ± 0.5	3.5 ± 1.0
Isobutyrate	1.1 ± 0.1	0.8 ± 0.1	0.3 ± 0.03	0.8 ± 0.1	0.5	0.4	0.1	0.3
Butyrate	15.7 ± 2.1	15.0 ± 2.1	4.7 ± 0.7	12.6 ± 1.7	11.5 ± 2.8	8.2 ± 2.0	3.5 ± 0.8	6.9 ± 1.7
Isovalerate	1.0 ± 1.4	1.0 ± 1.4	0.3 ± 0.4	0.8 ± 1.1	0.9	0.7	0.3	0.5
Valerate	1.8 ± 1.8	1.8 ± 1.8	0.5 ± 0.5	1.4 ± 1.4	0.0	0.0	0.0	0.0
Isocaproate	2.4 ± 0.7	2.3 ± 0.7	0.7 ± 0.2	1.8 ± 0.5	1.7 ± 0.7	1.2 ± 0.5	0.5 ± 0.2	1.0 ± 0.4
Caproate	19.2 ± 0.2	18.3 ± 0.2	5.4 ± 0.06	14.4 ± 0.2	6.6 ± 0.6	4.7 ± 0.5	1.8 ± 0.2	3.7 ± 0.4
sTotal	59.1 ± 5.4	56.1 ± 5.2	18.3 ± 1.8	48.8 ± 4.8	36.0 ± 7.2	25.7 ± 5.1	11.5 ± 2.3	22.9 ± 4.6

was observed in the fermentation without pH control, likely due to the reduced acidogenic activity caused by too low pH.

At pH 5.5, the kinetics of organic acids production was characterized by an initial stage of lactate accumulation (peaking at 11.5 g/L) and, in a lesser extent, acetate throughout the first 30 h of the process, followed by a stage of lactate and acetate consumption and butyrate production for the next 50–55 h of fermentation. On the contrary, the kinetics of organic acids production at pH 6.0 and 6.5 were characterized by an initial accumulation and consumption of lactate during the first 24 h, concomitantly with the production of acetate, butyrate and hydrogen. Residual concentrations of isobutyrate (<0.25 g/L) were also recorded. Lactate accumulation was slightly higher at pH 6.0 compared to pH 6.5, with an average concentration of 3.5 and 3.1 g/L, respectively. However, the most favourable pH in terms of BR was 6.5 (Fig. 2). Interestingly, the cumulative production of hydrogen plateaued with the depletion of lactate, suggesting the occurrence of LD-DF. Metabolically, hydrogen can be produced not only from carbohydrates but also via lactate-utilizing pathways co-producing butyrate or acetate [51]. The lactate flux toward acetate could explain the high amounts of acetate accumulated at pH 6 and 6.5. However, homoacetogenic acetate production should not be ruled out. According to Eq. (4), the acetate produced by homoacetogenesis ($Ac_{homoacet}$) at a fixed TS of 5 % accounted for 52.3, 22.5 and 23.6 % for pH 5.5, 6.0, and 6.5, respectively. A comparative analysis between the theoretical and experimental hydrogen production was conducted considering the concentrations of organic acids measured and the stoichiometry of hydrogen-producing and hydrogen-utilizing pathways (Eq. (8)–(11)). In general, the theoretical amounts of hydrogen agreed well with those recorded experimentally.

$$CH_3CH(OH)COOH \rightarrow 0.5CH_3CH_2CH_2COOH + CO_2 + H_2 + 0.5H_2O \quad (8)$$

$$CH_3CH(OH)COOH \rightarrow 0.5\,CH_3COOH + 0.5CH_3CH_2OH + CO_2 + H_2 \quad (9)$$

$$CH_3CH(OH)COOH + 0.5CH_3COOH \rightarrow 0.75CH_3CH_2CH_2COOH + CO_2 + 0.5H_2 + 0.5H_2O \quad (10)$$

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \quad (11)$$

On the other hand, low concentrations of formate peaked at 0.1–0.3 g/L but were further consumed concomitantly with lactate at pH 6.0 and 6.5. The role of formate in DF remains unclear to date. Its fate can be mediated by microbial lysis into CO_2 and hydrogen [10,52]. Interestingly, propionate was gradually produced after lactate depletion at 24 h of fermentation, reaching a final concentration of 2.4 and 2.1 g/L at pH 6.0 and 6.5, respectively (Fig. 2). Propionate production at neutral pH values has been reported in the literature [10,44]. Notably, no propionate accumulation was observed at pH 5.5, which agrees with previous research [15,41,53,54]. The onset of propionic-type fermentation is undesirable since it may imply the consumption of hydrogen, which corresponds to the reduction of hydrogen concentration in the acidogenic off-gas observed after lactate depletion. In this context, Cappai et al. (2014) [43] also observed an accumulation of acetate and butyrate within the first hours of fermentation, followed by propionate production when hydrogen production ceased at pH values between 6.0 and 7.0. These authors also observed a residual ethanol production under all conditions tested, which did not occur in the present study (data not shown). This implies that the operational conditions and inoculum herein used prevented the onset of ethanol-type fermentation. In addition, the occurrence of MCCAs was observed only at pH values of 6.0 and 6.5. Caproate and isocaproate were produced at low concentrations (≤1.0 g/L) from hour 45 onwards, alongside with residual concentrations of isovalerate.

The production kinetics for organic acids at a fixed pH of 6.5 were similar regardless of the TS content tested. The highest acidogenic activity was attained at 7.5 % TS (Table 2). However, a lower accumulation of lactate per VS fed (likely mediated by its rapid consumption within the first 24 h of fermentation) was recorded at a TS content of 7.5 %, which was approximately 30 % lower compared to the assays conducted at a TS content of 5 and 10 %. Thus, the highest hydrogen production observed at 7.5 % TS was correlated to a lower accumulation (or higher consumption) of lactate. Indeed, hydrogen production ceased when lactate was depleted. In this context, the fact that a superior hydrogen production was positively correlated with a reduced accumulation of lactate, but it ceased with the depletion of lactate, reinforces the hypothesises of the synergistic coupling between lactate producers (LAB) and lactate-utilizing HPB previously outlined in section 3.1. Additionally, higher concentrations of butyrate (8.7 g/L), caproate (8.7 g/L), and especially of acetate (10.7 g/L), were accumulated at a TS content of 7.5 %, compared to the assays conducted at TS contents of 5 % (77.6 %, 974 % and 26.8 % higher, respectively) and 10 % (83.2 %, 289 % and 62.8 % higher, respectively). $Ac_{homoacet}$ was estimated to be 23.6, 34.9 and 29.6 % in the assays conducted at a TS content of 5, 7.5 and 10 %, respectively. However, as mentioned earlier, it should be taken into account that the lactate flux may be exclusively directed toward acetate or butyrate, both leading to hydrogen production [51]. Finally, the conversion of FW into caproate has been reported to be mediated by lactate-based chain elongation, a pathway that could occur in this study [55]. Overall, the metabolic pathways herein observed strongly suggest the occurrence of LD-DF as the major hydrogen-producing route.

3.3. Biochemical potential tests

The acidogenic effluents obtained at the best operational conditions (pH and TS content) maximizing hydrogen production were subjected to BMP tests to elucidate the potential enhancement in the extent and rate of methanization due to the LD-DF process. After 36 days of methanization, the final methane yields from unfermented FW and from 24 and 48 h dark-fermented FW accounted for 346.5, 403.4 and 494.7 NmL CH_4/g VS added, respectively (Fig. 3 and Table 3). Therefore, the LD-DF of FW enhanced the extent of methane formation by 16.4 and 42.8 % after 24 and 48 h of fermentation, respectively. On the other hand, the maximum volumetric methane production rates, *Rmax,* were estimated to be 74.2, 68.0 and 92.8 NmL CH_4/h for unfermented FW and from 24 and 48 h dark-fermented FW, respectively. Interestingly, the assay performed with FW fermented for 24 h entailed a decrease in *Rmax* by 8.4 % compared to the control, while an increase in *Rmax* by 25.1 % was recorded in the assays with FW fermented for 48 h (Table 3). The *lag* phase of FW methanized was not significantly impacted by the LD-DF process, with an increase in λ from 0.9 h for unfermented FW to 1.9 h, regardless of the fermentation period. Finally, the t_{95} in the tests with unfermented FW and 24 and 48 h dark-fermented FW was 13.7, 19.2 and 16.9 days, respectively.

The difference in composition between the unfermented FW and the fermented FW likely explain the different methane kinetics recorded. On the one hand, the non-fermented FW had a complex but energetically rich composition, composed of polysaccharides, proteins and triglycerides. On the other hand, the fermented substrates were characterized by the presence of high concentrations of organic acids, specifically butyrate and acetate in the case of the FW fermented for 24 h, and caproate, butyrate, isovalerate and isocaproate in the case of FW fermented for 48 h. Although carbohydrates removal was herein not measured, the decrease in VS recorded indicates a significant reduction in organic matter for the dark-fermented FW. Despite the LD-DF process entails an inherent energy loss in the FW due to energy assimilation in the form of biomass, decarboxylation and hydrogen emission, the fermentation process also implies the availability of energy in the form of organic acids, which explains the higher methane yields obtained in the fermented substrates (1.06 g COD of glucose, compared to 1.82 or 2.21 g COD of butyrate or caproate, respectively) [56].

The production of methane from raw, unfermented FW was slightly

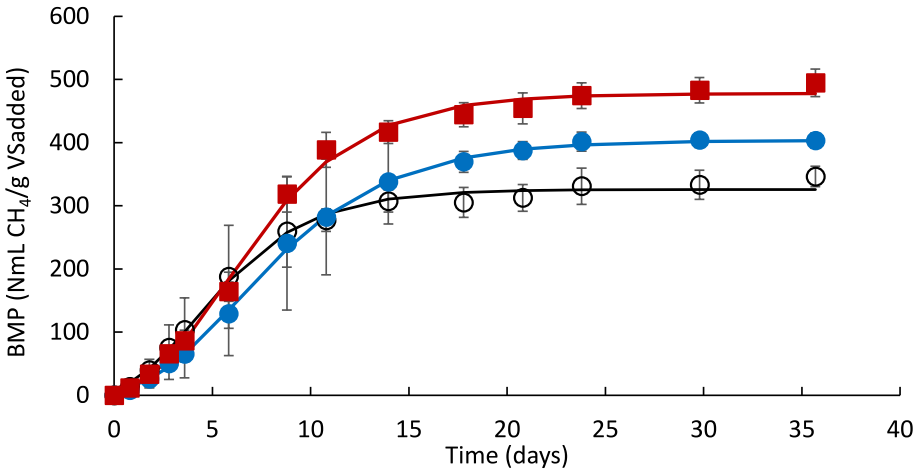

Fig. 3. Time course of methane production for unfermented FW (○) and FW fermented for 24 (●) and 48 (■) hours. Continuous lines indicate the modified Gompertz model prediction.

Table 3
Modified Gompertz kinetic model data obtained from the BMP assays with non-fermented FW (NFFW) and dark-fermented FW for 24 (F24) and 48 (F48) hours.

Condition	*P* (mL/g VS fed)	*Rmax* (mL/L-day)	*λ* (days)	R^2
NFFW	325.9	74.2	0.9	0.997
F24	403.7	68	1.9	0.999
F48	477.8	92.8	1.9	0.998

lower compared to the data in literature. Browne and Murphy (2013) [57] compiled data from a large variety of BMP assays and reported yields ranging from 314 to 529 NmL CH_4/g VS added, which were attributed to inoculum acclimation and the use of freshly collected FW. Similarly, Fisgativa et al. (2016) [58] obtained an average methane yield of 460.0 ± 87.6 NmL CH_4/g VS added. However, other authors have achieved similar methane yields to those recorded in this work: 364 NmL CH_4/g VS added [59] or 353 NmLCH_4/g VS added [60]. On the other hand, most studies reported in literature obtained higher methane yields (on a VS basis) using fermented FW. Nathao et al. (2013) [61] recorded a yield of 55 NmL H_2/g VS added and 94 NmL CH_4/g VS added in a two-stage FW fermentation, compared to the 82 NmL of CH_4/g VS added obtained in a single stage fermentation. Others, like de Gioannis et al. (2017) [62] reported a yield of 56.5 NmL H_2/g VS added and 392 NmL CH_4/g VS added in a two-stage anaerobic digestion process, compared to the 328.6 NmL CH_4/g VS added in a one stage anaerobic digestion, in an investigation carried out under comparable conditions of pH and FW composition that those used in the present study.

3.4. Global energy recovery

The best operational conditions applied in the LD-DF process of FW for hydrogen production (pH 6.5 and TS 7.5 %) supported a net energy recovery yield of 1.32 kJ/g VS fed (Table 4). Stoichiometrically, the maximum yield attainable by LD-DF is 4 mol of H_2/mol glucose equivalent [33]. Considering that the main component of FW with hydrogenogenic potential are carbohydrates [45,49,50], the yield obtained under the best process conditions was 1.58 mol H_2/mol glucose equivalent, followed by 1.24 and 1.22 mol H_2/mol glucose equivalent at 5 % and 10 % TS, respectively, and 1.13 and 0.85 mol H_2/mol glucose equivalent at pH 6.0 and 5.5, respectively. Such yields (based on hexose equivalent added at the beginning of test) implied a maximum hydrogen recovery efficiency of 40 %. In this context, Cappai et al. (2014) [43] obtained H_2 yields varying from 0.31 to 1 mol H_2/mol hexose from different mixtures of FW, where the lowest yields were attributed to the different carbohydrate composition of the FW tested. Similarly, Lee et al. (2014) [44] obtained a yield of 0.88 mol H_2/mol hexose at pH 7 and a yield of 1.63 mol H_2/mol hexose at pH 5.3 during the DF of FW.

Comparing the experimental energy recovered with the theoretical one (516.2 mL CH_4/g VS; 1305.3 kJ) calculated based on Eq. (3) [32], conventional anaerobic digestion of unfermented FW by methanogenesis resulted in an energy recovery ratio of 63.6 % (824.2 kJ) (Table 5). On the other hand, energy recovery ratios of 48.4 % (631.2 kJ) and 46.5 % (607.1 kJ), accounting for the sum of the H_2 and CH_4

Table 5
Energy recovery data from non-fermented FW (NFFW) and dark-fermented FW for 24 (F24) and 48 (F48) hours.

Condition	CH_4 yield (NmL/g VS fed)	Energy yield (kJ/g VS fed)	VS fed (g/L)	Total energy produced (kJ)	Recovery (%)
NFFW	346.5	12.17	72	876.2	67.1
F24	403.4	14.17	38.2	631.9	48.4
F48	494.7	17.37	29.5	607.1	46.51

Total Energy Produced indicates the total energy recovered from the LD-DF + methanogenic process. *Recovery* indicates the percentage of energy recovered (H_2 + CH_4) compared to the theoretical maximum (%), based on Eq. (3).

Table 4
Energy recovery in the FW fermentation for H_2 production carried out at different pH and TS contents.

Condition		H_2 yield (mol H_2/mol hexose fed)	Energy yield (kJ/g VS fed)	Energy produced (kJ)	Recovery (%)
pH	uncontrolled pH	0	0	0	0
	5.5	0.85	0.71	33.53	21
	6.0	1.13	0.94	44.85	28
	6.5	1.24	1.03	48.96	31
TS (%)	7.5	1.58	1.32	94.87	40
	10	1.22	1.02	97.17	31

Recovery indicates the percentage of H_2 produced compared to the theoretical maximum.

produced (kJ equivalents), were estimated in the FW fermented for 24 and 48 h, respectively. Interestingly, the H_2 produced from the 24 and 48 h dark-fermented FW represented 14.3 % (90.4 kJ) and 15.6 % (94.5 kJ) of the total energy recovered, which is considerably lower compared to the high VS removal (55.4 %) from this DF process. Overall, the LD-DF process resulted in an increase in the methane yield, but it did not offset the losses in VS that occurred during the acidogenic phase.

4. Conclusions

The influence of two key process parameters, pH and TS content, on the performance of LD-DF of FW was investigated. The results obtained showed a marked influence of pH and TS content on the hydrogen production performance, leading to a superior hydrogen production when approaching more neutral pH values and increasing the concentration of solids (although a TS content of 10 % became detrimental to the process likely due to mass transfer limitations associated to the inefficient mixing provided by magnetic stirring at that solids load). The target parameters also exerted a notable effect on the organic acid profile, where a clear correlation was observed between lactate production and consumption, and hydrogen production. A pH of 6.5 and TS of 7.5 % was identified as the best experimental condition tested in this study, supporting a hydrogen yield of 103.4 NmL H_2/g VS fed and a maximum volumetric hydrogen production rate of 13.3 NL H_2/L-d, with the concomitant production of 8.7 g/L of caproate. Altogether, the LD-DF process shows a high potential for co-producing hydrogen and organic acids including MCCAs from FW. Alternatively, the effluent generated by the LD-DF can be valorised through the production of renewable biogas.

CRediT authorship contribution statement

Lois Regueira-Marcos: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Octavio García-Depraect:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Raúl Muñoz:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fuel.2022.127238.

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