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ABSTRACT

This study aims to investigate the impact of utilizing lactic acid fermentation (LAF) as storage method of food waste (FW) prior to dark fermentation (DF). LAF of FW was carried out in batches at six temperatures (4°C, 10°C, 23°C, 35°C, 45°C, and 55°C) for 15 days followed by biological hydrogen potential (BHP) tests. Different storage temperatures resulted in different metabolites distribution, with either lactate or ethanol being dominant (159.2±20.6 mM and 234.4±38.2 mM respectively), but no negative impact on BHP (averaging at 94.6±25.1 mL/gVS). Maximum hydrogen production rate for stored FW improved by at least 57%. Microbial analysis showed dominance of lactic acid bacteria (LAB) namely *Lactobacillus* sp., *Lactococcus* sp., *Weisella* sp., *Streptococcus* sp. and *Bacillus* sp. after LAF. *Clostridium* sp. emerged after DF, co-existing with LAB. Coupling LAF as a storage method was demonstrated as a novel strategy of FW management for DF, for a wide range of temperatures.

KEYWORDS : biohydrogen, dark fermentation, lactic acid fermentation, mixed culture, energy

1. INTRODUCTION

Hydrogen is seen as the fuel of the future, credited to its clean combustion and high energy density (141.9 MJ/kg), significantly higher than other common fossil fuels: methane (55.5 MJ/kg), ethane (51.9 MJ/kg), gasoline (47.5 MJ/kg), diesel (44.8 MJ/kg) and methanol (20 MJ/kg) (Nikolaidis and Poullikkas, 2017). In 2020, demand for hydrogen was 90 Mt; mostly generated from fossil fuels: 79% from dedicated hydrogen production plants and 21% as by-product from gasoline refining processes (International Energy Agency, 2021). Biochemical conversion processes such as dark fermentation (DF) have among the lowest environmental impacts, especially on global warming and acidification potentials as compared to other methods (Aydin and Dincer, 2022). FW being rich in macronutrients, have previously been investigated as resources for bioenergy (H_2, CH_4) using combinations of biological methods, and for other commodity compounds such as organic acids (Sufficiency et al., 2022). However, the main drawback of using FW as a substrate is that organic carbon losses can occur during storage and transportation due to spontaneous fermentation (Parthiba Karthikeyan et al., 2018). To address this issue, LAF has long been used to preserve food, crops or produce silage for animal feed and employed as a storage strategy prior to anaerobic digestion with the objective to maximize the biomethane potential of substrate (Villa et al., 2020). LAF has also been recently proposed to produce high value-added molecules such as lactic acid from FW (Chenebault et al., 2022). However, there is no research on utilizing lactic acid fermentation as a storage method prior to DF for biohydrogen production. This is understandably so as LAB was viewed as detrimental to hydrogen producing bacteria (HPB), leading researchers to steer the process away from LAB proliferation (García-Depraect et al., 2021). However, this is

not always the case (Castelló et al., 2020) and the interaction between LAB and HPB warrants further investigation (García-Depraect et al., 2021). In mixed cultures, other microorganisms than HPB are present such as methanogens, propionic fermenters and homoacetogens that can directly consume hydrogen in reactors (Castelló et al., 2020). Microbial communities' composition and activities are therefore crucial to determine the metabolic pathways occurring in DF and subsequently affecting the biohydrogen yield. In this light, the novelty of the present investigation is to study the coupling of LAF as a storage method for DF and its effect on the microbial communities developed. More particularly, storage temperatures will be investigated. This approach opens new possibilities of managing organic substrates for hydrogen production for a wide range of climates.

2. MATERIALS AND METHODS

2.1 Substrate composition and characteristics

This study used the same FW investigated by Magdalena et al. (2023) and Noguer et al. (2022), consisted of a mixture of minced beef (15%), yoghurt (10%), mixed berries (15%), breaded fish (10%), French fries (20%), mixed vegetables of broccoli, long beans, carrots, and potatoes (7.5%), mixed carrots (7.5%), bread (15%), representing 62% carbohydrates, 22% proteins and 16% lipids on total mass basis. The biodegradable COD of the food waste was 1.28 ± 0.2 gCOD/VS, determined using near infrared spectrometry (Noguer et al., 2022b). Particle size was 215.6±273.2 μm (measured in the range 0–2000 μm), analyzed using granulometer (Beckman Coulter LS200). sCOD (21.2 g/L) and tCOD (122.8 g/L) of substrate was determined using commercial kits (Lovibond, Germany). Each component of FW was frozen at -20°C to

prevent changes in composition over time. It was blended to 10% TS prior to fermentation using a hand blender (Dynamic MiniPro) at high speed (13,000 rpm) for a total blending time of 10 minutes with Milli-Q water, and directly used for LAF storage.

2.2 Lactic acid fermentation as storage

700 g of the substrate (at 10% TS) were introduced into a 1 L glass bottle reactor with custom neck for sampling. The headspace was purged with nitrogen gas to ensure anaerobic condition. Reactors were placed at 4°C, 10°C, 23°C, 35°C, 45°C and 55°C, in triplicate for 15 days, to simulate wide range of application temperatures. 15 days was selected based on study of FW lactic acid fermentation by Daly et al. (2020). No stirring was done to simulate static storage, except prior to sampling for homogenization. Five mL of liquid samples were periodically collected using syringe through custom sampling neck, every day for the first three days and every three to five days until the last day, centrifuged and the biomass and supernatant stored at -20°C. Total volume withdrawn was on average 45 ± 7 mL per reactor, well below 10% of the total volume.

2.3 Biohydrogen potential (BHP) assays

After 15 days, the stored food waste (SFW) was fermented in DF assays according to standardized protocol (Carrillo-Reyes et al., 2020) to assess the influence of the storage temperatures on the BHP. 20 g of SFW from each storage bottles were transferred into individual 600 mL glass bottles. For each bottle, 20 mL of 1 M MES (2-(N-Morpholino)ethanesulfonic acid) were added as buffer to maintain the pH, and final pH was adjusted to pH 6 using 8 M NaOH. Finally, Milli-Q water was added to reach a total working volume of 0.2 L. The bottle headspace was purged with nitrogen gas to

ensure anaerobic condition and placed at 37°C in a water bath. The bottles were connected to a micro gas chromatograph for continuous online gas production measurement and analysis. At the end of the BHP test, 5 mL of liquid were sampled for metabolite and microbial analysis.

2.4 Analytical methods

2.4.1 Determination of total solids and volatile solids content

Total solid (TS) and volatile solid (VS) were determined by drying samples at 105°C (Memmert) for 24 hours followed by 550°C (Nabertherm) for 3 hours. TS and VS contents of stored FW were corrected based a method proposed by Kreuger et al. (2011) to consider volatilized volatile fatty acids (VFAs) during drying at 105°C.

2.4.2 Metabolite analysis by High Performance Liquid Chromatography

pH was measured for each liquid sample using a pH-meter (WTW inoLab pH7110) equipped with an electrode probe (WTW Sentix 41). Liquid samples were centrifuged at 13,400 rpm for 15 minutes and the supernatant was analyzed for quantification of organic acids such as lactate, VFAs and ethanol (Noguer et al., 2022b). Samples were acidified with H_2SO_4 0.1M and filtrated through 0.2 μm nylon filter (Fisherbrand). Metabolites were analyzed using HPLC (Thermo Scientific Dionex Ultimate 3000) equipped with a refractive index detector (ERC RefractoMax 520). HPLC analysis was performed at a flow rate of 0.6 L/min with a column (Aminex HPX-87H Ion exclusion) at 50°C equipped with a protective pre-column (Bio-Rad Micro-Guard Cation H^+).

2.4.3 Biogas Analysis by gas chromatography

During LAF storage experiments, headspace pressure was measured using handheld digital manometer (Keller LEO2 adapted with hypodermic needle). Gases were sampled using gas-tight syringe and 150 µL were analyzed by gas chromatography (Clarus 580, Perkin Elmer) equipped with RtQbond column (for H_2, O_2, N_2 and CH_4 gases) and a RtMolsieve column (for CO_2), coupled with thermal conductivity detector and Argon as the carrier gas (Chenebault et al., 2022). During DF experiments, the BHP bottles were automatically sampled every two hours by a micro-gas chromatograph (SRA l-GC 3000) equipped with a PoraPlot U (PPU) 8 m column at 70°C, 20 psi with helium as carrier gas for CO_2 analysis, and Molsieve 5A 10 m column at 80°C, 30 psi with argon as carrier gas for H_2, O_2, N_2, CH_4 analysis (Noguer et al., 2022b).

2.5 Data analysis

2.5.1 Assessment of biogas production

The volume of gas production was calculated using the Equations 1 and 2:

$$\Delta N(n) = \left[y(n)P(n)\frac{Vh}{RT}\right] - \left[y(n-1)P(n-1)\frac{Vh}{RT}\right] \quad (1)$$

$$\Delta V(n) = \Delta N(n)\frac{RT_0}{P_0} \quad (2)$$

Where ΔN(n) is the change of number moles of gas produced at sampling time n, y(n) is the gas composition (% v/v) at a sampling time n, P(n) is headspace pressure at sampling time n, Vh is the headspace volume, R is the gas constant (8.314 J/mol·K), T is the temperature of sampled gas, ΔV(n) is the volume of gas produced from the previous measurement, T_0 is 273.15 K and P_0 is 10^5 Pa. The cumulative hydrogen production is the sum of ΔV obtained throughout the fermentation period.

2.5.2 Reaction Advancement

The reaction advancement (RA) was defined as the amount of substrate converted to gas or metabolites over the initial amount of COD, as expressed in Equation 3.

$$RA = \frac{Total\ metabolites\ in\ gCOD/L}{Initial\ biodegrability\ of\ substrate\ in\ g\ of\ biodegradable COD/L} \times 100\% \quad (3)$$

2.5.3 Model fitting and Statistical Analysis

Hydrogen production data obtained from the BHP reactors were fitted into the modified Gompertz equation to model gas production growth as proposed by Lay et al. (1996):

$$H_t = P_m \cdot exp\left\{-exp\left[\frac{R_m \cdot e}{P_m}(\lambda - t) + 1\right]\right\} \quad (4)$$

where H_t is the cumulative biohydrogen production, P_m is the maximum biohydrogen production, R_m is the maximum biohydrogen production rate, λ is the lag phase, t is the fermentation time and e is Euler's number (Equation 4).

ANOVA statistical test was applied to verify statistical significance between the results. When significant difference was observed, a Tukey's test was applied, obtaining Tukey's honestly significant difference (HSD) to differentiate the statistically different groups of results (Dauptain et al., 2020). ANOVA and Tukey's tests were carried out using Microsoft Excel Data Analysis Tool and the online tool Astatsa, respectively. Principal Component Analysis (PCA) was done using RStudio with factoextra library.

2.6 Microbial Community Analysis

The biomass recovered after centrifugation was utilized for microbial analysis. DNA extraction was performed using FastDNA SPIN kit for soil following manufacturer's instructions (MP biomedicals, LCC, California, USA). Amplification was done on the V3 – V4 region of the 16S rRNA using universal primers. The PCR mix consisted of MTP Taq DNA Polymerase (Sigma-Aldrich, Germany) (0.05 u/µL) with enzyme

buffer, forward (344F: ACGGRAGGCAGCAG) and reverse (802R: TACCAGGGTATCTAATCCT) primers (0.5 mM), dNTP (0.2 mM), sample DNA (5-10 ng/μL) and water to reach a final volume of 50 μL. 35 cycles of denaturation at 95°C for 1 minute, annealing at 65°C for 1 min and elongation at 72°C for 1 min were carried out using thermal cycler (Mastercycler, Eppendorf, Germany). After 35 amplification cycles, a final elongation was performed for 10 minutes at 72°C. Verification of PCR amplifications was done using 2100 Bioanalyzer (Agilent, USA). For sequencing of reaction, GenoToul platform (Toulouse, France http://www.genotoul.fr) using Illumina Miseq sequencer (2 x 300 pb paired-end run) was utilized. Raw sequences were analyzed using Mothur version 1.48.0 for reads cleaning, assembly and quality checking and SILVA release 132 was used for alignment and as taxonomic outlines. For visual processing, Microsoft Excel together with Power Query tool were utilized. Genera with less than 1% relative abundance were grouped as "others".

3. RESULTS AND DISCUSSION

3.1 Lactic Acid Fermentation as a Storage Method of FW prior to Dark Fermentation

3.1.1 Storage temperatures determined the fermentation pathways and the dynamics of metabolite production

Gas production, metabolite concentration and pH were measured throughout 15 days of storage. LAF was dominant, indicated by CO_2 production and no detection of H2 and CH_4. Figure 1 (a-f) shows the trends of metabolite accumulation within the 15 days of FW storage at 4°C, 10°C, 23°C, 35°C, 45°C and 55°C. Lactate accumulation was observed at all temperatures, albeit at different rates and final concentrations. At 4°C and 10°C (Figure 1, a-b), lactate production started after day seven and three

respectively, reaching a final concentration of 39.6±2.4 mM and 57.7±1.3 mM, respectively. For all other temperatures (Figure 1 c, d, e, f), lactate accumulated before day 2, to reach a final concentration of 136.7±2.6 mM at 23°C, 159.2±20.6 mM at 35°C, 66.9±10.9 mM at 45°C, and 22.4±7.0 mM at 55°C. The slower fermentation rate at low temperatures was attributed to a low microbial growth or microbial activity. Consistently, slow production of organic acids from FW was reported at low temperature (15°C) as compared to higher temperatures of 25°C and 35°C (Daly et al., 2020). In the present study, the final concentration of lactate at 35°C (159.2±20.6 mM) was significantly lower compared to Daly et al., (2020), where concentration reached 936 mM after 15 days at the same TS concentration of 10%. This was likely caused by i) the different composition of substrate, which was only carbohydrate-rich residues as compared to the present study, which considered a mixture of carbohydrates, proteins and lipids, and ii) the different amounts of soluble COD over total COD ratio, which was 0.62 (3.6 times higher) compared to 0.173 in the present work. Moreover, Tang et al. (2016) obtained a concentration of 32.8 g/L (364.1 mM) of lactate from FW fermentation at 37°C and 7% TS, more than twice from this study, likely due to the use of pH control at pH 6 and continuous stirring which were not implemented in the present investigation. More consistent with the present study, Chenebault et al, (2022) showed an accumulation of 177 mM (15.9±0.5 g/L) of lactate from FW after 8 days of incubation at 20 % TS, double the TS content of this study. In this study, carried out under very similar conditions (no external inoculation and no pH control) a concentration of 157±8 mM (15.1±0.8 g/L) of lactate was achieved for the same duration of experiment. The yield was 0.09±0.0 g/gTS compared to this study at 0.15±0.0 g/gTS. Yang et al., (2022) reported similar lactate concentration and yield of

168±7 mM and 0.15±0.0 g/gTS, respectively when LAF of FW was carried out at 37°C, 10%TS and no pH control.

The initial average pH of the FW was 4.9±0.1 and after 15 days of storage, the pH decreased to a final pH value of 3.6±0.3. The final pH correlated with the final lactate concentration, as lactate has a pKa value lower than the other organic acids (Latham et al., 2019). Daly et al. (2020) reported a similar result where storage fermentation of carbohydrate-rich FW exhibited a final pH ranging between 3 and 4 for temperatures of 15°C, 25°C and 35°C. Such low pH might explain why the lactate pathways were mostly favored during storage and the absence hydrogen-producing fermentation, as previously shown to be governed by pH values (Daly et al., 2020). LAF is strongly favored at pH values between 3 and 5.5, and if the pH level is higher, butyrate-type fermentation pathway is then promoted (Daly et al., 2020).

3.1.2 Contribution of homolactic and heterolactic pathways during LAF

Lactate production during storage could occur through either the homolactic (only lactate) or heterolactic (lactate + ethanol + acetate) pathways and was determined from the ethanol produced during fermentation. Ethanol production was detected at all temperatures except at 55°C, where ethanol was only detected at day 2 (Figure 1, f). At 4°C, ethanol production started after eight days of fermentation, and continuously increased to reach 11.2±2.4 mM at day 15. At 10°C, ethanol was detected after three days of storage alongside lactate production. Then ethanol surpassed lactate concentration between day 10 and 14, and increased up to 88.4±35.3 mM of ethanol on the last day, suggesting a shift in the metabolic pathway. A similar trend was observed

249 at 23°C, with a final ethanol concentration of 234.4±38.2 mM. A stoichiometric balance
250 analysis suggested that three possible metabolic pathways could have occurred:

251 i) Homolactic pathway: $\mathrm{Glucose} \rightarrow 2\mathrm{Lactate}$ (5)

252 ii) Heterolactic pathway: $\mathrm{Glucose} \rightarrow \mathrm{Lactate} + \mathrm{Ethanol} + \mathrm{CO_2}$ (6)

253 iii) Ethanolic pathway: $\mathrm{Glucose} \rightarrow 2\mathrm{Ethanol} + 2\mathrm{CO_2}$ (7)

254 In heterolactic fermentation (Eq. 6) 1 mol of lactate is produced along with 1 mol of
255 ethanol and 1 mol of CO_2. At 10°C and 23°C, 1.5 and 1.7 mol of ethanol and 2 and 1.9
256 mol of CO_2 (44.4 mM and 123.3 mM) respectively were produced per mol of lactate.
257 Because there were more than 1 mol per mol of lactate, additional mol of ethanol
258 possibly came from a concurrent and direct ethanolic pathway (Eq. 7). At 4°C, 35°C
259 and 45°C, 0.3 mol, 0.4 mol and 0.5 mol of ethanol were produced per mol of lactate,
260 suggesting a dominance of homolactic fermentation. Consistently, Yang et al., (2022)
261 reported 30.7±2.4 mM (1.414±0.112 g/L) of ethanol produced alongside 168.1±7.1 mM
262 (15141±636 mg/L) of lactate at 37°C and 10%TS, equivalent to 0.01 mol of ethanol per
263 mol of lactate, indicating a largely dominant homolactic pathway. Chenebault et al.,
264 (2022) reported relatively small amounts of ethanol as compared to lactate during LAF
265 of FW at 24°C and 35°C, with 0.13±0.02 and 0.21±0.01 mol of ethanol per mol of
266 lactate, respectively, suggesting also a dominance of the homolactic pathway.
267 Information is scarce on LAF of FW at low temperatures (4°C to 24°C) as most of the
268 studies focused on the maximization of the lactate production usually performed at
269 mesophilic and thermophilic temperatures. At 55°C, no ethanol was detected except
270 briefly on the second day with a small amount of 4.2±7.3 mM, suggesting also
271 dominant homolactic fermentation or ethanol volatilization.

As secondary intermediate, acetate can be produced in heterolactic fermentation, along with ethanol and CO_2 via the 6-phosphogluconate pathway (de Angelis and Gobbetti, 2016) or through lactate degradation by LAB (Bühlmann et al., 2022). In all storage temperatures, acetate was produced except at 55°C. At 4°C and 10°C, acetate accumulated between day 6 to 8 and 3 to 7, respectively and remained constant at 14 mM until day 15. At 23°C, 35°C and 45°C storage temperatures, acetate was produced before day 2 and remained constant until day 15, with an average concentration of 16.2±5.6 mM. Yang et al. (2022), reported acetate was produced in a relatively small amount compared to lactate when pH was below 5.5. However, after the pH was increased to 6, the amount of acetate increased with the reduction of lactate and the production of hydrogen gas, indicating a clear shift to butyric fermentation.

In addition, small amounts of propionate (2±1.7 mM) were detected at 4°C after 14 days of fermentation, but not on the final day. Apart from 4°C, propionate was also detected at 23°C at day 12 (4.2±3.7 mM), and its concentration increased until 5.3±4.8 mM on the final day. Theoretically, 1 mol of carbohydrate and H_2 are stoichiometrically converted to 2 mol of propionate.

The temperatures of 4°C, 35°C, 45°C and 55°C were more favorable to homolactic fermentation whereas the temperatures of 10°C and 23°C showed a combination of metabolic pathways and a more diverse metabolites distribution (Figure 2). Meanwhile, lactate production increased from the low storage temperature of 4°C (4.6±0.2 gCOD/L) and 10°C (6.0±0.2 gCOD/L) to reach a maximal value at 35°C (15.3±2.0 gCOD/L). At higher temperature, a decrease was observed with 6.4±1.0 gCOD/L and 2.2±0.7 gCOD/L at 45°C and 55°C. Assessment of the RA indicated that significant amounts of the initial biodegradable COD of the FW were transformed after

15 days storage (Figure 2). The extent of RA was highest at 23°C accounting for 30±3% and followed by 35°C, 10°C, 45°C, 4°C and 55°C at 19±2%, 12±3%, 9±1%, 5±1%, 2±1%. In similar study, Chenebault et al. (2022) achieved only 12.9% of RA at 35°C, lower than this study. Daly et al. (2020) reached 25% and 56% RA for FW fermented at 15°C and 35°C, higher than this study attributable to higher activity of LAB.

3.1.4 Temperatures affect the dominant genera during storage

Figure 3 shows the microbial composition at genus level of the microbial communities present at the end of each storage temperature. At least 98.6% of the relative abundance were represented by eight major genera, regardless the temperature considered. *Lactobacillus* sp. was the main genus at 4°C, 23°C, 35°C, 45°C and in one sample at 55°C (relative abundance 72.3%, 81.5%, 99.0%, 46.4 %). *Lactobacillus* sp. is the largest genus within the group of LAB and is mainly used in food fermentation including dairy, bread, vegetables, and meat (de Angelis and Gobbetti, 2016).

Streptococcus sp. was present especially at the temperatures of 4°C, 10°C and 23°C with an average relative abundance of 22 %, 57 % and 1 % respectively. *Streptococcus* sp. mainly produces lactate, but is also able to produce acetate, formate and ethanol under carbohydrate-restricted conditions (Gobbetti and Calasso, 2014). Nucleotide alignment using BLAST (Basic Local Alignment Search Tool) showed 98.59 % alignment with the species *Streptococcus thermophilus. Streptococcus thermophilus* is a food-grade bacteria usually used in the production of cheese and yoghurt (Liu et al., 2020), which might explain the presence in the storage reactors in this study, as yoghurt is one of the compositions of the FW.

Lactococcus sp., as LAB, was identified at 4°C, 10°C and 23°C with a relative abundance of 2.8%, 21.8% and 1.4% respectively. This genus is a homolactic fermenter that produces only lactate as metabolite (Issa and Tahergorabi, 2019), confirming the predominance of this pathway at low storage temperatures. BLAST showed 98.83 % alignment of this OTUs with *Lactococcus plantarum*, as closest relative. *Lactococcus plantarum* is a facultatively anaerobic, gram-positive, non-motile bacteria extracted from frozen peas and was reported to grow at 10°C but not 45°C (Schleifer et al., 1985). This was in-line with this study, where it had the highest relative abundance at 10°C and probably came from the long beans in the FW composition. *Lactococcus lactis* is usually found in raw dairy and *Streptococcus thermophilus* is used as a starter in dairy products, and subtle interactions between these two species are reported. For instance, *Lactococcus lactis* shows up to 20% increment of viable count when paired with *Streptococcus thermophilus* in a simulated cheese fermentation (Champagne et al., 2009). Although *Lactococcus lactis* is not present in this study, this particular interaction could also have occurred here between other species.

Bacillus sp. was observed as most abundant genus in two replicates of the storage reactors at 55°C. *Bacillus* are facultative anaerobes known to survive in a wide range of conditions including high temperatures and a wide range of pH (Jenson, 2014). At 55°C lactate was only produced in the first two days before stagnating, suggesting no growth of fermentation activity. OTU BLAST showed 99.06% similarity with *Lactiplantibacillus plantarum* for the first sample at 55°C, and 99.66% similarity with *Weizmannia coagulans* (formerly *Bacillus coagulans*) for the second and third samples. *Bacillus coagulans* was reported to be temperature resistant, having an optimal growth temperature between 35°C to 50°C (Wang et al., 2022).

343 *Weissella* as part of *Leuconostocaceae* family which is known to mostly have
344 heterofermentative LAB, which produces ethanol and CO_2 together with lactate
345 (Lonvaud-Funel, 2014). The two highest amounts of ethanol in FW stored at 10°C and
346 23°C (Figure 2) also had the highest relative abundance of *Weisella* at 3.8±1.2% and
347 10.9±2.2%. This was confirmed by a statistical correlation between the relative
348 abundance of *Weissella* sp. and ethanol concentration ($R2=0.9$, $p<0.05$).
349 *Pseudomonas* sp. was found in all triplicates at 45°C with a relative abundance
350 of 43.8%±29.7%. *Pseudomonas* sp. are strictly aerobic but some species are able to use
351 nitrate (which can naturally be found in food) as terminal electron acceptor and can
352 grow anaerobically (Dodd, 2014). *Pediococcus* sp. were also found, but at low relative
353 abundance with the highest relative abundance of 3.7±0.3% at 23°C. *Pediococcus* sp.
354 can be homolactic or heterolactic fermenters, with some strains producing equal amount
355 of lactate and ethanol from fermentation of xylose (Raccach, 2014).
356 Figure 4 visualized the correlation between process parameter and outcomes
357 (temperature, CO_2, VFAs and lactic acid), and microbial communities. CO_2 and ethanol
358 production were closely correlated to *Weissella* sp. and *Pediococcus* sp. due to their
359 heterolactic fermentative pathway. RA was closely related to CO_2 production, attributed
360 to heterolactic fermentation. *Lactococcus* sp. and *Streptococcus* sp. were closely
361 correlated, further supporting the microbial communities relationship. The temperature
362 vector correlated closer to the genera *Pseudomonas sp* and *Bacillus sp* as both were
363 observed at the higher temperatures 45°C and 55°C. Consistently, lactate production
364 correlated with the abundance in *Lactobacillus* sp. *Lactococcus* sp. and *Streptococcus*
365 sp. despite being lactate fermenters were negatively correlated, probably due to their

366 dominance at only 10°C storage temperature. The narrow angle between their two
367 vectors aligns with assumption on relationship between these two genera.
368
369 3.2 Conversion of Stored Food Waste (SFW) to Hydrogen
370 *3.2.1 Biohydrogen potential not impacted by the storage temperature, except at 55*°C
371 BHP (Biological Hydrogen Potential) tests were carried out to evaluate the effect of the
372 storage temperatures on hydrogen potential. Table 1 shows the maximum production
373 (P_m), the maximum production rate (R_m) and the lag phase (λ) obtained from the
374 modified Gompertz equation fitted to the experimental data. The average maximum
375 hydrogen production for SFW was 94.6±25.1 mL/gVS. No significant difference of
376 maximum hydrogen production was observed between the fresh FW (control) and the
377 SFW pre-fermented at 4°C, 10°C, 23°C, 35°C and 45°C (ANOVA, P>0.3), for an
378 average hydrogen yield of 83.9±10.5 mLH_2/gVS. This result clearly evidenced that
379 storing FW in LAF at these temperatures for 15 days did not impact the biohydrogen
380 potential. It is noteworthy that, in this study, the initial concentrations of metabolites in
381 the BHP reactors were ranging between 1.8 to 17.4 mM for lactate and 0 to 2.1 mM for
382 acetate, that were far below the inhibitory threshold of 50 mM as reported by (Noguer et
383 al., 2022a). In addition, hydrogen production at these temperatures was comparable to
384 the values reported (65±12 mL/gVS) by (Noguer et al., 2022b), using the same
385 substrate. However, biohydrogen production in the present study was slightly higher
386 than the one reported in Elbeshbishy et al., (2011), where 42 mL/gVS of hydrogen were
387 produced from untreated FW. Interestingly, these authors investigated the effect of FW
388 pretreatments such as heat, ultrasonic, combination of ultrasonic and heat, and
389 combination of ultrasonic and base, and they reported very similar yields (70 mL/gVS,

90 mL/gVS, 78 mL/gVS and 67 mL/gVS) to those obtained in the present study after pretreatment, suggesting a difference in carbohydrate accessibility.

As an exception, at 55°C, the hydrogen yield was significantly higher at 141.4±34.6 mL/gVS or 68% compared to the average of other storage temperatures (Tukey's HSD, $p<0.05$). Such increase of hydrogen performances was probably due to heat-pretreatment effect at thermophilic temperature.

In contrast with the other parameters, the maximum biohydrogen production rate (R_m) significantly improved after FW storage. All SFW showed higher R_m averaging at 62.4±16.2 mL/gVS·d than fresh food waste (FFW) at 29.3±11.1 mL/gVS·d. The difference in production rate in this case, despite the similar yields was attributed to a higher accessibility of the organic matter due to preliminary degradation during storage.

3.2.2 Organic acids produced during LAF are mostly consumed or converted in DF

Table 2 shows the concentrations of initial and final metabolites in the BHP reactors. Lactate was not detected at the end of the process, suggesting that lactate was converted to hydrogen or other metabolites. Similarly, ethanol was not detected at the end of the BHP process, either being volatilized or converted to other metabolites in a chain elongation process. Few pathways were reported for lactate and ethanol consumption under anaerobic conditions as summarized by Hillion et al. (2018). The presence of caproate in the BHP reactor of SFW stored at 35°C indicated the occurrence of chain elongation probably through butyrate and ethanol or butyrate and lactate producing caproate. For all conditions, butyrate and acetate were the major metabolites which is consistent with the literature (Ghimire et al., 2015).

3.2.3 Conversion to Hydrogen by Different Genera for SFW Stored at Different Temperatures

Figure 5 shows the composition of the microbial communities in terms of relative abundance at genus level, at the end of the BHP assays. As the BHP tests were performed on LAF residues, four genera present in the LAF reactors remained in the DF reactors: *Lactobacillus* sp., *Lactococcus* sp. and *Streptococcus* sp., from the *Lactobacillaceae* family, and *Bacillus* sp., from the *Bacillaceae* family. After DF, nine additional genera emerged, the most dominant being *Clostridium* sp. with 26.8±4.4%, 35.8±4.3%, 41.5±4.4%, 52.7±0.3%, 41.3±2.8%, 24.6±3.8% and 29.0±12.2% of relative abundance for the SFW stored at 4°C, 10°C, 23°C, 35°C, 45°C and 55°C respectively. *Clostridium* sp. are considered as the genus having the most efficient HPB in DF (Castelló et al., 2020). The presence of both LAB and HPB in the DF reactors, indicated the ability of co-existence between them. This is in-line with recent studies that suggested the presence of positive relationship between LAB and HPB despite earlier studies suggesting the opposite (García-Depraect et al., 2021). The presence of both can even produce a positive relationship in the form of cross-feeding, or the former assisting hydrolysis for the later to produce hydrogen (García-Depraect et al., 2021). The emergence of the HPBs was here favored by the initial adjustment of the pH to 6, in the BHP test. These results evidenced that endogenous FW inoculum is sufficient in the case of coupling LAF and DF. Endogenous HPB found in FW were thus able to survive the low pH of LAF for at least 15 days. This also resonated with previous observations made by Dauptain et al. (2020) who reported that indigenous bacteria were as effective at producing biohydrogen as exogenous thermal-pretreated inoculum. Interestingly,

436 *Enterococcus* sp., a LAB not significantly present after storage, emerged after DF with
437 5.6±2.2% and 10.4±3.3% relative abundance at 10°C and 55°C storage.
438 Figure 6 shows that hydrogen production positively correlated with butyrate, a
439 common metabolic pathway in DF. Weirdly, *Clostridium* sp. despite being known as
440 main contributor in hydrogen DF production showed inverse correlation with hydrogen
441 accumulation and was closely correlated to acetate production. It can therefore be
442 assumed that some hydrogen was consumed to produce acetate, as some *Clostridium* sp.
443 were reported to be efficient homoacetogens in DF, consuming 4 mol of hydrogen gas
444 to produce 1 mol of acetate (Castelló et al., 2020). The emergence of *Enterococcus* sp.
445 and the positive correlation with hydrogen production might indicate a strong HPB
446 activity of *Enterococcus* sp. when BHP tests are fed with pre-stored FW.
447 Although storage temperatures was shown to have no significant impact on BHPs
448 (except at 55°C), the impact of other storage parameters such as concentration and
449 storage duration during LAF on DF should be investigated in future work.
450

451 **4. CONCLUSIONS**

452 Temperature was crucial in determining the metabolite profile developed during
453 storage, where ethanol was the most abundant product at lower temperatures whilst
454 lactate was promoted at higher temperatures. LAF of FW was demonstrated to be
455 efficient to store transitorily the food waste without affecting the hydrogen potential
456 (94.6±25.1 mL/gVS on average). LAF as storage increased the maximum production
457 rate in subsequent BHP. Although LAB dominated after storage at low pH (3-4),
458 indigenous HPB emerged during DF when conditions were more favorable at pH 6 and
459 37°C, where both co-existed with one another.

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6. REFERENCES

1) Aydin, M.I., Dincer, I., 2022. A life cycle impact analysis of various hydrogen production methods for public transportation sector. Int J Hydrogen Energy 47, 39666–39677. https://doi.org/10.1016/j.ijhydene.2022.09.125
2) Bühlmann, C.H., Mickan, B.S., Tait, S., Batstone, D.J., Mercer, G.D., Bahri, P.A., 2022. Lactic acid from mixed food waste fermentation using an adapted inoculum: Influence of pH and temperature regulation on yield and product spectrum. J Clean Prod 373, 133716. https://doi.org/10.1016/j.jclepro.2022.133716
3) Carrillo-Reyes, J., Buitrón, G., Moreno-Andrade, I., Tapia-Rodríguez, A.C., Palomo-Briones, R., Razo-Flores, E., Aguilar-Juárez, O., Arreola-Vargas, J., Bernet, N., Braga, A.F.M., Braga, L., Castelló, E., Chatellard, L., Etchebehere, C., Fuentes, L., León-Becerril, E., Méndez-Acosta, H.O., Ruiz-Filippi, G., Tapia-Venegas, E.,

Trably, E., Wenzel, J., Zaiat, M., 2020. Standardized protocol for determination of biohydrogen potential. MethodsX 7. https://doi.org/10.1016/j.mex.2019.11.027

4) Castelló, E., Nunes Ferraz-Junior, A.D., Andreani, C., Anzola-Rojas, M. del P., Borzacconi, L., Buitrón, G., Carrillo-Reyes, J., Gomes, S.D., Maintinguer, S.I., Moreno-Andrade, I., Palomo-Briones, R., Razo-Flores, E., Schiappacasse-Dasati, M., Tapia-Venegas, E., Valdez-Vázquez, I., Vesga-Baron, A., Zaiat, M., Etchebehere, C., 2020. Stability problems in the hydrogen production by dark fermentation: Possible causes and solutions. Renewable and Sustainable Energy Reviews 119. https://doi.org/10.1016/j.rser.2019.109602

5) Champagne, C.P., Gagnon, D., St-Gelais, D., Vuillemard, J.C., 2009. Interactions between Lactococcus lactis and Streptococcus thermophilus strains in Cheddar cheese processing conditions. Int Dairy J 19, 669–674. https://doi.org/10.1016/j.idairyj.2009.06.002

6) Chenebault, C., Moscoviz, R., Trably, E., Escudié, R., Percheron, B., 2022. Lactic acid production from food waste using a microbial consortium: Focus on key parameters for process upscaling and fermentation residues valorization. Bioresour Technol 354. https://doi.org/10.1016/j.biortech.2022.127230

7) 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 Sustain Chem Eng 8, 13934–13944. https://doi.org/10.1021/acssuschemeng.0c03161

8) Dauptain, K., Trably, E., Santa-Catalina, G., Bernet, N., Carrere, H., 2020. Role of indigenous bacteria in dark fermentation of organic substrates. Bioresour Technol 313, 123665. https://doi.org/10.1016/j.biortech.2020.123665

9) de Angelis, M., Gobbetti, M., 2016. Lactobacillus SPP.: General Characteristics ☆. Reference Module in Food Science. https://doi.org/10.1016/b978-0-08-100596-5.00851-9

10) Dodd, C.E.R., 2014. Pseudomonas: Introduction. Encyclopedia of Food Microbiology: Second Edition 3, 244–247. https://doi.org/10.1016/B978-0-12-384730-0.00282-2

11) Elbeshbishy, E., Hafez, H., Dhar, B.R., Nakhla, G., 2011. Single and combined effect of various pretreatment methods for biohydrogen production from food waste. Int J Hydrogen Energy 36, 11379–11387. https://doi.org/10.1016/j.ijhydene.2011.02.067

12) 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. https://doi.org/10.1016/j.biortech.2020.124595

13) Ghimire, A., Frunzo, L., Pirozzi, F., Trably, E., Escudie, R., Lens, P.N.L., Esposito, G., 2015. A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products. Appl Energy 144, 73–95. https://doi.org/10.1016/j.apenergy.2015.01.045

14) Gobbetti, M., Calasso, M., 2014. Streptococcus: Introduction. Encyclopedia of Food Microbiology: Second Edition 3, 535–553. https://doi.org/10.1016/B978-0-12-384730-0.00324-4

15) Hillion, M. lou, Moscoviz, R., Trably, E., Leblanc, Y., Bernet, N., Torrijos, M., Escudié, R., 2018. Co-ensiling as a new technique for long-term storage of agro-industrial waste with low sugar content prior to anaerobic digestion. Waste Management 71, 147–155. https://doi.org/10.1016/j.wasman.2017.10.024

16) International Energy Agency, 2021. Global Hydrogen Review 2021. Global Hydrogen Review 2021. https://doi.org/10.1787/39351842-en

17) Issa, A.T., Tahergorabi, R., 2019. Milk Bacteria and Gastrointestinal Tract: Microbial Composition of Milk. Microbial Composition of Milk., Dietary Interventions in Gastrointestinal Diseases: Foods, Nutrients, and Dietary Supplements. Elsevier Inc. https://doi.org/10.1016/B978-0-12-814468-8.00022-3

18) Jenson, I., 2014. Bacillus: Introduction. Encyclopedia of Food Microbiology: Second Edition 1, 111–117. https://doi.org/10.1016/B978-0-12-384730-0.00018-5

19) Kreuger, E., Nges, I., Björnsson, L., 2011. Ensiling of crops for biogas production: Effects on methane yield and total solids determination. Biotechnol Biofuels 4, 1–8. https://doi.org/10.1186/1754-6834-4-44

20) Latham, K.G., Ferguson, A., Donne, S.W., 2019. Influence of ammonium salts and temperature on the yield, morphology and chemical structure of hydrothermally carbonized saccharides. SN Appl Sci 1, 1–13. https://doi.org/10.1007/s42452-018-0055-2

21) Lay, J.-J., Li, Y.-Y., Noike, T., 1996. Effect Of Moisture Content And Of Chemical Fermentation Nature On Methane Characteristics Solid Wastes. J. Environ. Syst. and Eng. 1, 101–108.

22) Liu, G., Qiao, Y., Zhang, Y., Leng, C., Chen, H., Sun, J., Fan, X., Li, A., Feng, Z., 2020. Metabolic Profiles of Carbohydrates in Streptococcus thermophilus During

554 pH-Controlled Batch Fermentation. Front Microbiol 11, 1–11.
555 https://doi.org/10.3389/fmicb.2020.01131
556 23) Lonvaud-Funel, A., 2014. Leuconostocaceae Family, Second Edi. ed,
557 Encyclopedia of Food Microbiology: Second Edition. Elsevier.
558 https://doi.org/10.1016/B978-0-12-384730-0.00185-3
559 24) Magdalena, J.A., Pérez-Bernal, M.F., Bernet, N., Trably, E., 2023. Sequential
560 dark fermentation and microbial electrolysis cells for hydrogen production: Volatile
561 fatty acids influence and energy considerations. Bioresour Technol 374.
562 https://doi.org/10.1016/j.biortech.2023.128803
563 25) Nikolaidis, P., Poullikkas, A., 2017. A comparative overview of hydrogen
564 production processes. Renewable and Sustainable Energy Reviews 67, 597–611.
565 https://doi.org/10.1016/j.rser.2016.09.044
566 26) Noguer, M.C., Escudié, R., Bernet, N., Eric, T., 2022a. Populational and
567 metabolic shifts induced by acetate, butyrate and lactate in dark fermentation. Int J
568 Hydrogen Energy 47, 28385–28398. https://doi.org/10.1016/j.ijhydene.2022.06.163
569 27) Noguer, M.C., Magdalena, J.A., Bernet, N., Escudi, R., 2022b. Enhanced
570 Fermentative Hydrogen Production from Food Waste in Continuous Reactor after
571 Butyric Acid Treatment. Energies (Basel) 15, 1–18.
572 https://doi.org/https://doi.org/10.3390/en15114048
573 28) Parthiba Karthikeyan, O., Trably, E., Mehariya, S., Bernet, N., Wong, J.W.C.,
574 Carrere, H., 2018. Pretreatment of food waste for methane and hydrogen recovery: A
575 review. Bioresour Technol 249, 1025–1039.
576 https://doi.org/10.1016/j.biortech.2017.09.105

29) Raccach, M., 2014. Pediococcus. Encyclopedia of Food Microbiology: Second Edition 3, 1–5. https://doi.org/10.1016/B978-0-12-384730-0.00247-0

30) Schleifer, K.H., Kraus, J., Dvorak, C., Kilpper-Bälz, R., Collins, M.D., Fischer, W., 1985. Transfer of Streptococcus lactis and Related Streptococci to the Genus Lactococcus gen. nov. Syst Appl Microbiol 6, 183–195. https://doi.org/10.1016/S0723-2020(85)80052-7

31) Sufficiency, E., Qamar, S.A., Ferreira, L.F.R., Franco, M., Iqbal, H.M.N., Bilal, M., 2022. Emerging biotechnological strategies for food waste management: A green leap towards achieving high-value products and environmental abatement. Energy Nexus 6, 100077. https://doi.org/10.1016/j.nexus.2022.100077

32) Tang, J., Wang, X., Hu, Y., Zhang, Y., Li, Y., 2016. Lactic acid fermentation from food waste with indigenous microbiota: Effects of pH, temperature and high OLR. Waste Management 52, 278–285. https://doi.org/10.1016/j.wasman.2016.03.034

33) Villa, R., Ortega Rodriguez, L., Fenech, C., Anika, O.C., 2020. Ensiling for anaerobic digestion: A review of key considerations to maximise methane yields. Renewable and Sustainable Energy Reviews 134, 110401. https://doi.org/10.1016/j.rser.2020.110401

34) Wang, L., Wang, J., Du, L., Fang, X., Liao, Z., 2022. Application of Weizmannia coagulans in the medical and livestock industry. Ann Microbiol 72. https://doi.org/10.1186/s13213-022-01687-3

35) 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

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1) Figure 1: Accumulation of metabolites during 15 days of storage at temperature of a) 4°C, b) 10°C, c) 23°C, d) 35°C, e) 45°C and f) 55°C. Metabolites not detected or below quantification limit were not plotted.
2) Figure 2: Cumulated final metabolites concentration of stored food waste (SFW) according to the storage temperature and extent of the reaction advancement
3) Figure 3: Relative abundance at genera level of the microbial communities after 15 days storage of FW at different temperatures (triplicates are individually presented)
4) Figure 4: PCA analysis of process outcomes (gas and metabolites) and microbial communities with process parameter (storage temperatures) for LAF storage of FW'
5) Figure 5: Relative abundance at genera level at the end of conversion of FW to hydrogen in DF assays
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2) Table 2: Initial and final concentration of metabolites in the BHP reactors

Table 1: Variable values Pm, Rm and λ from Modified Gompertz Equation Fitting. Superscript letters represent Tukey's test results with values sharing the same letters are not statistically different.

Substrate storage temperature (°C)	P_m, maximum production (mL/gVS)	R_m, maximum production rate (mL/gVS·d)	λ, lag phase (d)
Fresh FW	76.8±11.9^{a}	29.3±11.1^{a}	0.8±0.1^{cd}
4	80.0±5.7^{a}	46.1±5.9^{ab}	0.4±0.1^{a}
10	89.3±0.5^{a}	53.4±10.1^{ab}	0.7±0.0^{bc}
23	79.0±5.1^{a}	71.8±17.8^{b}	1.1±0.0^{e}
35	94.0±19.9^{a}	69.5±14.3^{b}	0.5±0.1^{ab}
45	83.4±2.1^{a}	57.8±10.6^{ab}	0.6±0.0^{ab}
55	141.4±34.6^{b}	75.6±20.6^{b}	0.9±0.1^{d}

629 *Table 2: Initial and final concentration of metabolites in the BHP reactors*

	Intial					Final				
FW storage temperature (°C)	Lactate (gCOD/L)	Acetate (gCOD/L)	propionate (gCOD/L)	Ethanol (gCOD/L)	Total (gCOD/L)	Lactate (gCOD/L)	Acetate (gCOD/L)	Butyrate (gCOD/L)	Caproate (gCOD/L)	Total (gCOD/L)
4	0.4 ± 0.0	0.1 ± 0.0	-	0.1 ± 0.0	0.6 ± 0.0	-	1.2 ± 0.2	2.8 ± 0.4	-	4.0 ± 0.6
10	0.6 ± 0.0	0.1 ± 0.0	-	0.8 ± 0.3	1.5 ± 0.4	-	0.9 ± 0.1	3.9 ± 0.1	-	4.7 ± 0.1
23	1.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	2.3 ± 0.4	3.8 ± 0.4	-	0.5 ± 0.5	3.2 ± 0.1	-	3.8 ±0.4
35	1.5 ± 0.2	0.1 ± 0.0	-	0.6 ± 0.1	2.3 ± 0.3	-	0.7 ± 0.0	3.2 ± 0.8	0.7 ± 1.2	4.5 ± 0.5
45	0.6 ± 0.1	0.1 ± 0.0	-	0.3 ± 0.0	1.0 ± 0.1	0.1 ± 0.2	-	3.4 ± 0.8	-	3.5 ± 0.7
55	0.2 ± 0.1	-	-	-	0.3 ± 0.1	-	-	3.6 ± 0.1	-	3.6 ± 0.1

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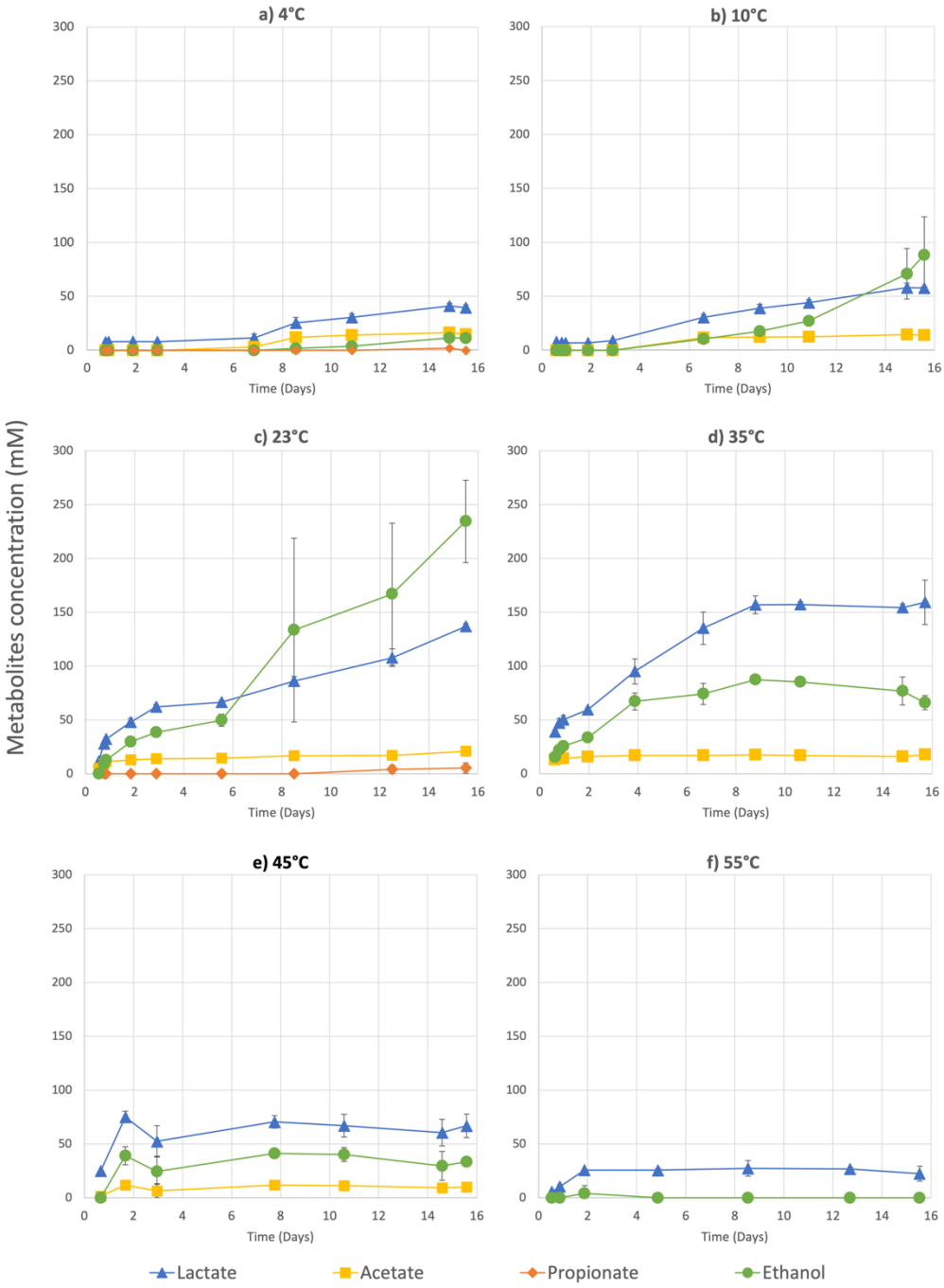

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633 *4°C, b) 10°C, c) 23°C, d) 35°C, e) 45°C and f) 55°C. Metabolites not detected or below*
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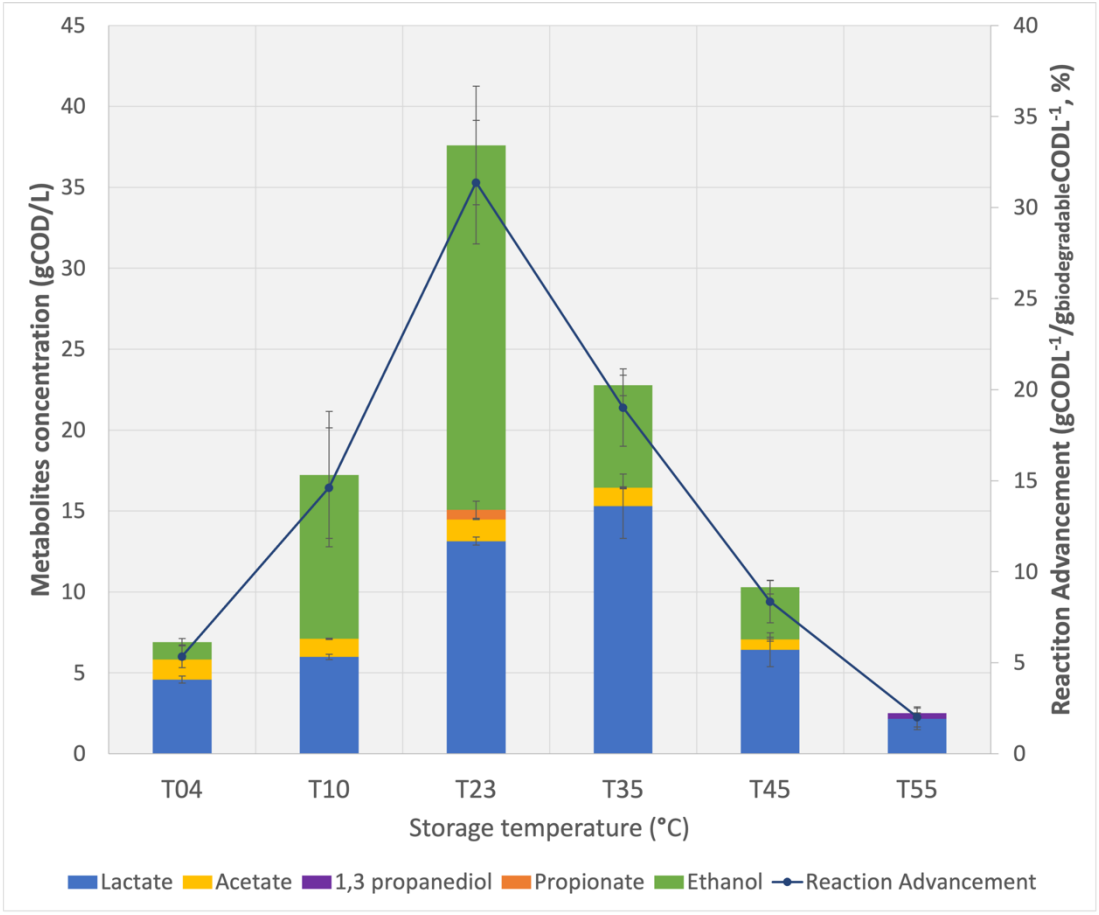

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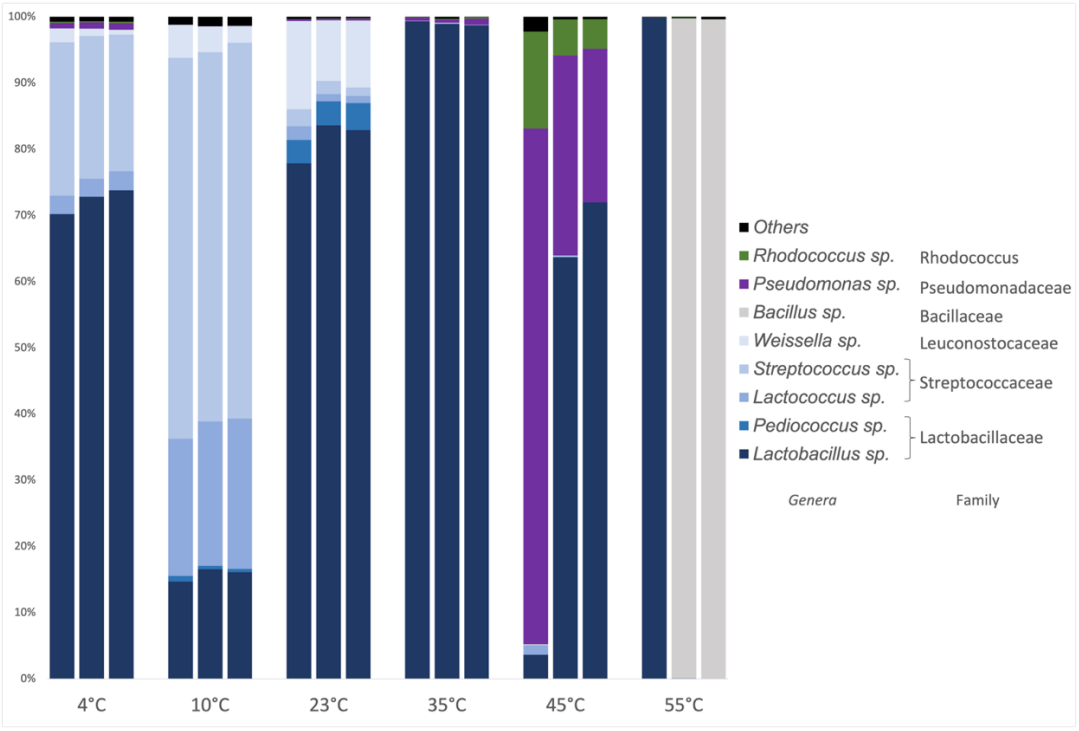
638 *according to the storage temperature and extent of the reaction advancement.*



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640 *Figure 3: Relative abundance at genera level of the microbial communities after 15*
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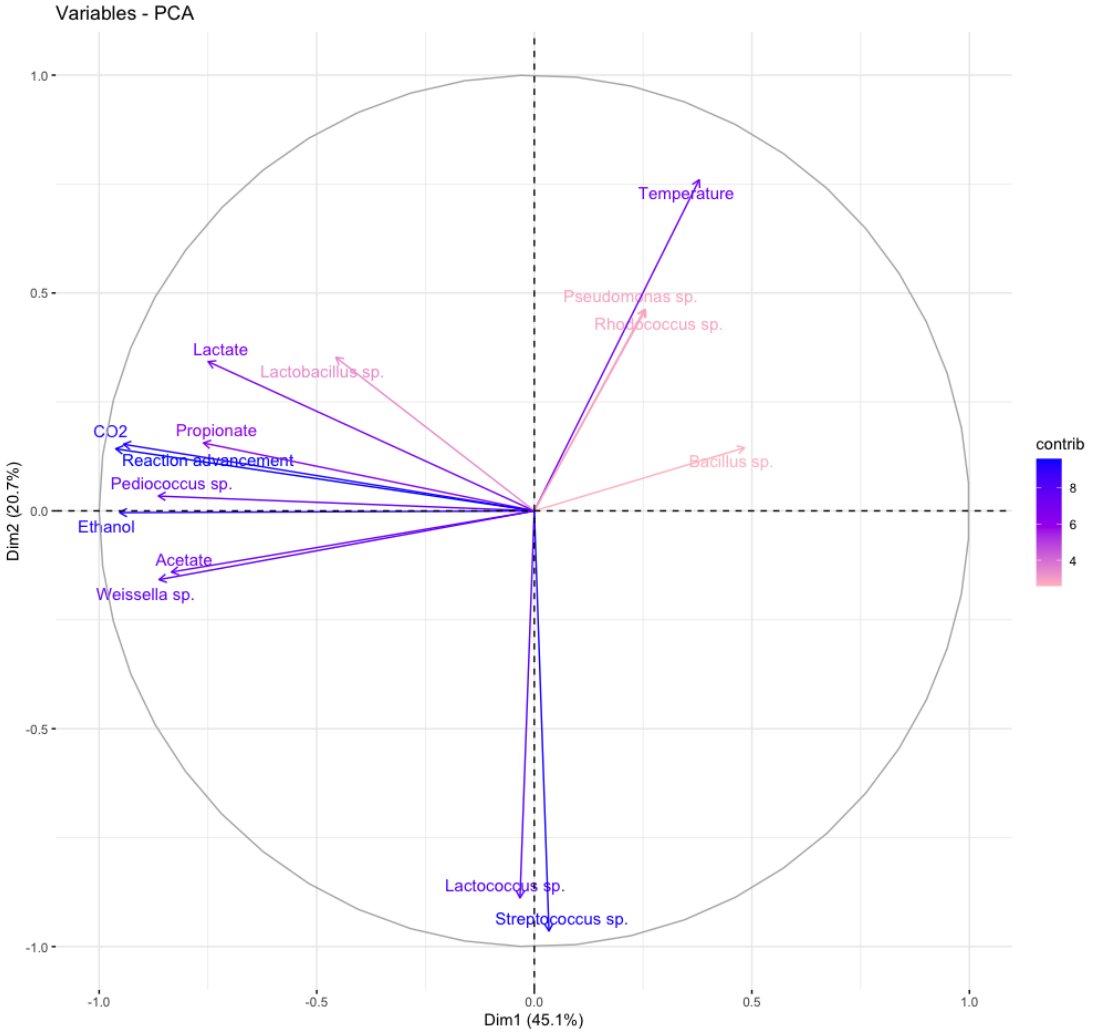

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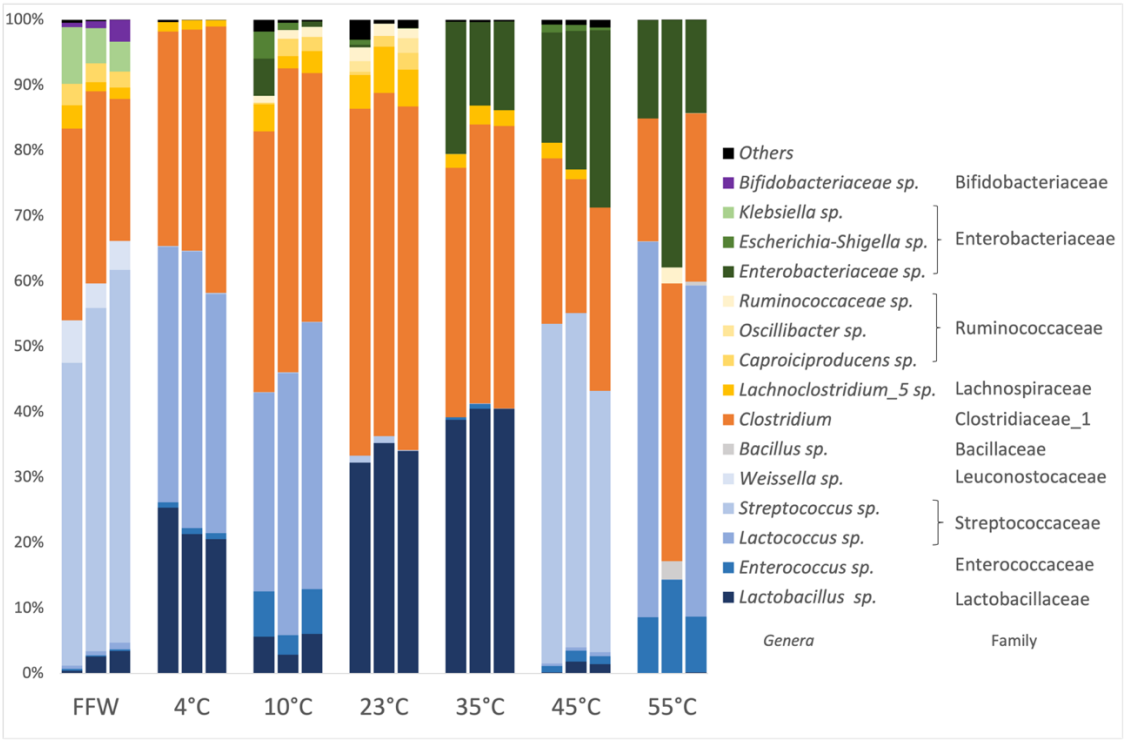

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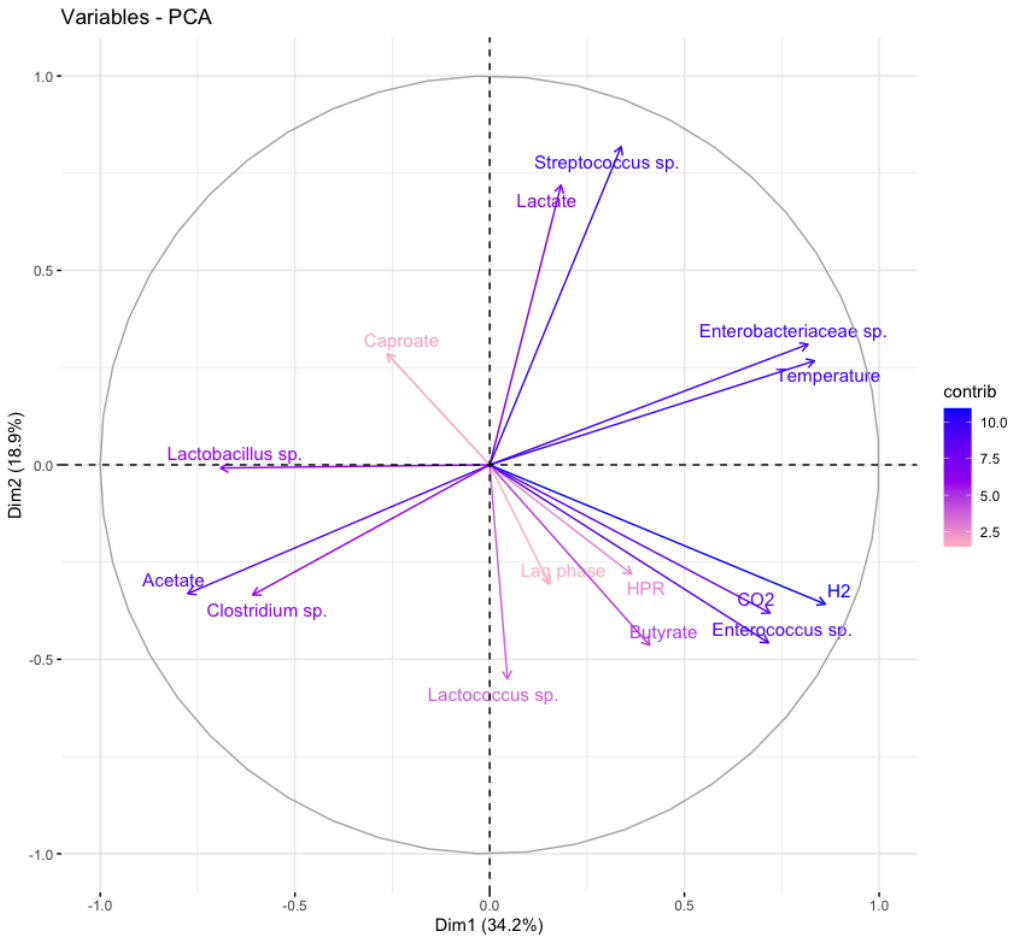

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