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Two-stage anaerobic digestion of fruit and vegetable waste: optimization of dark fermentation through thermal pretreatment and co-digestion with sugar-rich wastewater

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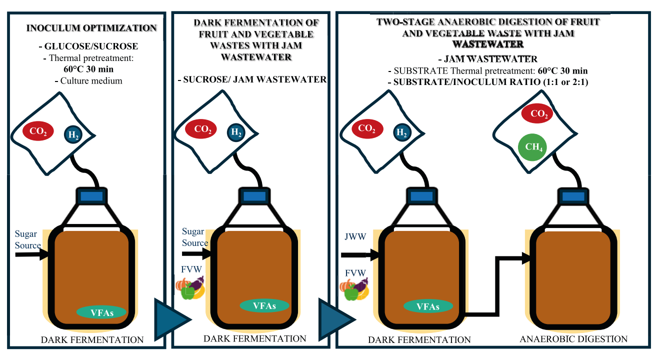
HIGHLIGHTS

- Thermal pretreatment of inoculum enhances dark fermentation efficiency.
- Culture medium can be avoided and jam wastewater can act as sugar source.
- Two-stage anaerobic digestion on fruit and vegetable waste is presented.
- Hydrogen and volatile fatty acids yields align with literature values.
- Methane yield is higher than traditional anaerobic digestion.

GRAPHICAL ABSTRACT



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ABSTRACT

Fruit and vegetable waste (FVW) poses environmental challenges but can be valorized via dark fermentation (DF) and two-stage anaerobic digestion (TSAD) to produce H_2 and CH_4. This study evaluated the effects of culture medium and thermal pretreatment of inoculum in DF, testing various sugars and jam wastewater (JWW) as a renewable sugar source. Thermal pretreatment at 60 °C for 30 min enriched H_2-producers, while culture medium had no significant effect. Glucose and sucrose allow H_2 yield of 6.5 and 45.8 N mL/g_{VS}, respectively. JWW proved suitable for DF of both raw and pretreated FVW, achieving H_2 yields of 67.1 and 99.5 N mL/g_{VS}, respectively. Volatile fatty acids after DF indicated active fermentation pathways reaching 7.2 g/L in JWW_FVW_1:1. In the TSAD, CH_4 production reached 184 N mL/g_{VS}, surpassing conventional single-stage digestion. These results demonstrate the potential of TSAD systems using FVW and JWW for efficient simultaneous H_2 and CH_4 production.

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1. Introduction

The depletion of fossil fuels is a critical global issue; hence, alternative and renewable energy sources have grown in interest, especially biomass waste valorization. Biomass residues are renewable and carbon–neutral, making them a promising resource for sustainable energy production and climate change mitigation (Mazzanti et al., 2025).

Food waste is a major contributor to biomass waste, largely driven by the rising global demand for food. In the EU, approximately 59 million tons of food waste is generated annually (Eurostat, 2022). Half of household food waste is linked to the consumption of fresh fruits and vegetables, while a significant portion also arises from agricultural and industrial sectors (De Laurentiis et al., 2018; Martínez-Mendoza et al., 2023). Fruit and vegetable waste (FVW) is typically managed through landfilling or composting. However, landfilling has been increasingly restricted due to CH_4 emissions and leachate which contaminates soil with organic matter, salts, pollutants, and heavy metals. Composting has been proposed as an alternative for managing easily biodegradable waste like FVW but can lead to leachate production, odor and greenhouse gases emissions, posing several environmental challenges (Brown et al., 2013; Esparza et al., 2020; García-Rández et al., 2025).

Exploiting FVW into bioenergy and biochemicals offers a promising solution, aligning with Circular Economy principles and Sustainable Development Goals 2030 (European Commission, 2015). FVW can be valorized through biological conversion into energy carriers: hydrogen (H_2) and methane (CH_4). Its high moisture and carbohydrate content, along with beneficial compounds, make it ideal for microbial conversion (Martínez-Mendoza et al., 2023). H_2 can be generated through dark fermentation (DF), an anaerobic process producing H_2– and CO_2-rich gas along with a digestate rich in volatile fatty acids (VFAs). DF is simple and energy-efficient but limited by low yields and a low technological readiness level (≤ 5). Two-stage anaerobic digestion (TSAD) addresses these drawbacks by separating conventional anaerobic digestion into two steps: DF, which produces a VFAs-rich digestate, followed by anaerobic digestion, converting VFAs into CH_4. TSAD offers simultaneous H_2 and CH_4 production, better biomass utilization, and higher CH_4 yields than single-stage anaerobic digestion (AD). The final digestate retains valuable nutrients (carbon, nitrogen, phosphorus) for further use. However, TSAD is complex, requiring tailored conditions per stage and suppression of methanogens during DF (Mazzanti et al., 2025). To achieve this, pretreatment of the inoculum is necessary to eliminate methanogens, encouraging the proliferation of H_2-producing bacteria. Thermal pretreatment was selected as one of the preferred methods for enhancing H_2-producers activity, as well as chemical treatments. Thermal technique involves microbial heating at temperatures between 60–120 °C during 15–240 min. Ravindran et al. (2010) observed the highest H_2 production at 95 °C (1.93 mol/mol$_{Glucose}$), followed by 105 °C (1.92 mol/mol$_{Glucose}$) while untreated inoculum allowed a H_2 yield of 0.54 mol/mol$_{Glucose}$, suggesting higher temperatures favor sporulation of key strains. Similarly, Hidalgo et al. (2023) showed that while temperature had a limited impact on H_2 yield, residence time was critical; 30 min improved biogas production at 80 °C (275 mL/g$_{Glucose}$ compared to 240 mL/g$_{Glucose}$ in the unpretreated case) and 100 °C (275 mL/g$_{Glucose}$ compared to 245 mL/g$_{Glucose}$ in the unpretreated case), whereas 60 min was more effective at 60 °C (285 mL/g$_{Glucose}$ compared to 240 mL/g$_{Glucose}$ in the unpretreated case). Chemical pretreatment involves the modification of inoculum pH to stimulate H_2-producers development. Strong acids such as HCl, H_2SO_4, and HNO_3 can be added to the culture until a certain pH is reached. Lee et al. (2009) evaluated the use of the three acids while Hidalgo et al. (2023) explored the use of HCl and H_2SO_4. Both studies concluded that HCl is the most effective acid for chemical pretreatment. Although thermal pretreatment is a straightforward process, it does not always fully eliminate H_2-consuming microorganisms. Acid pretreatments are generally simple, cost-effective, and efficient, but they are associated to pH adjustment post-treatment, the potential formation of undesirable by-products that may require disposal, and the need for periodic repetition to maintain efficacy (Bundhoo et al., 2015). While some studies have identified chemical pretreatment as the most effective, others have highlighted heat shock treatment as the best strategy for eliminating methanogens and enhancing H_2 production (Al-Haddad et al., 2023; Chen et al., 2021a). Considering the goals of waste reduction and minimizing the use of non-renewable chemicals, thermal pretreatment appears to be the more environmentally preferable option.

Glucose is commonly employed as a model sugar source in DF studies due to its high bioavailability. Mizuno et al. (2000) conducted DF in a 2.3 L continuous stirred-tank reactor (CSTR) at 35 °C and pH maintained at 6. A culture medium containing 10 g/L of glucose was added, resulting in a H_2 content of 53 % in the biogas and an H_2 yield of 110 mL/g$_{carbohydrates}$. Similarly, Kumar and Das (2000) performed DF in 50 mL rubber-stoppered conical flasks at 36 °C over 10 h, using 1 % glucose. This setup achieved a H_2 yield of 270 mL/g$_{carbohydrates}$. Beyond glucose, other sugars have also been explored for DF. Fang et al. (2002) investigated the use of sucrose (2–12.15 g/L) in a 3 L fermenter at 26 °C, maintaining the pH at 5.5. The resulting biogas contained 63 % H_2, with an H_2 yield of 280 mL/g$_{carbohydrates}$, demonstrating the viability of sucrose as an effective sugar source for DF. The use of commercial sugars such as glucose and sucrose poses economic challenges for large-scale biogas plants. Among alternatives, jam industry wastewater represents a novel and mostly underexplored waste. Mohan and Sunny (2008) demonstrated 90 % COD reduction and methane production from jam wastewater (JWW) in batch systems. Ruffino and Zanetti (2017) achieved 0.329 Nm^3 of CH_4 per kg of volatile solids (VS) in 6 L reactors, validating its potential in AD. However, JWW has not yet been explored as a sugar source in DF or TSAD.

This study investigates JWW as a renewable sugar source for DF and TSAD of FVW. Inoculum activity was first assessed with simple sugars, with and without culture medium, and thermal pretreatment was tested to enhance performance. DF was then carried out by replacing commercial sugars with JWW, while TSAD explored the effects of substrate-to-inoculum ratio and substrate pretreatment on H_2 and CH_4 yields. Results demonstrate that JWW, an underexplored byproduct, can effectively support DF and TSAD, providing energy recovery while reducing freshwater use. The integration of FVW and JWW thus represents a sustainable circular economy approach for biomass waste valorization.

2. Materials and methods

2.1. Substrates and inoculum characterization

The inoculum employed in DF and AD was the mesophilic digestate of cow-agricultural sludge (CAS) provided by "Cascina La Speranza" (Fossano, Cuneo, Italy). FVW was collected from household residue (mass composition of 75 % vegetables and 25 % fruits) and chopped through a kitchen blender. JWW was supplied by the agricultural cooperative "Agricopecetto" (Pecetto Torinese, Turin, Italy) as the water employed to wash jam production equipment. Both FVW and JWW were frozen at −18 °C after their collection to avoid natural decomposition. Then they were defrosted before being fed into the fermentation system. JWW, FVW and inoculum characterization are presented in Table 1. All the experiments have been performed with the same lot of JWW, FVW and inoculum to ensure the reproducibility and comparability of the achieved results.

2.2. Inoculum thermal pretreatment

In the present study, inoculum was thermally pretreated at 60 °C for 30 min to eliminate H_2-consumers and favor sporulation of H_2-producers. Time and temperature were chosen in accordance with literature results (Hidalgo et al., 2023; Ravindran et al., 2010). Inoculum was heated in the oven covered by aluminum foils to avoid significant water

Table 1
Chemical and physical characterization of inoculum, FVW and JWW.

	Inoculum	FVW	JWW
TS [%]	7.00 ± 0.00	7.50 ± 0.01	0.94 ± 0.00
VS/TS [%]	72.73 ± 0.02	92.95 ± 0.02	95.67 ± 0.03
N [%]	3.18 ± 0.35	2.60 ± 0.29	0.96 ± 0.02
C [%]	37.43 ± 1.26	39.41 ± 4.33	52.86 ± 3.98
H [%]	5.17 ± 1.28	5.54 ± 0.44	6.75 ± 0.28
S [%]	0.44 ± 0.16	0.50 ± 0.04	0.31 ± 0.04
O [%]	53.78 ± 2.72	51.96 ± 5.03	39.14 ± 4.23

*Average of 3 samples. FVW = Fruit and vegetable waste, JWW = jam wastewater, TS = total solids, VS = volatile solids, N = nitrogen, C = carbon, H = hydrogen, S = sulfur, O = oxygen.

evaporation from the samples.

2.3. Process setup and operative condition

DF and AD were performed in 250 mL Pyrex glass bottles (Duran, Germany) with a working volume of 80 % at 35 °C. The heating was controlled by a 55 L thermostatic water-bath (Julabo-Corio-C, Merck, Germany). Reactors were operated in batch feeding mode with 6 % of total solids and shaken manually to keep the mixture homogeneous inside the reactor. Each reactor was sealed with a two-port cap. Through one of the ports, anaerobic conditions were assured by purging N_2 directly inside the biomass to change the volume of the reactor three times and closing the port. The second port was connected to a 1 L Teddlar gas bag where biogas was collected. The duration of each assay depended on biogas or H_2 production: testing was stopped when daily H_2 (for DF tests) and biogas (for AD tests) production was less than 1 % of the overall production recorded during the period of preparation (VDI 4630, 2006). The experimental campaign consists of three main experiments and all the campaigns were studied in replicates. The pH of each reactor was measured at the beginning and at the end of the experiments. In the first test, no pH adjustment was applied in order to monitor how the pretreated inoculum behaved in terms of natural pH variation during dark fermentation. In the second and third experiments, when necessary, HCl was added to the reactors to lower pH to around 6–7 in DF. AD pH was maintained between 7 and 8.

In the first experiment campaign, eight configurations were tested to investigate the role of the inoculum in the DF. In detail, the utilization of the inoculum thermally pre-treated (to inhibit the methanogenic consortia), the addition or not of a medium culture, and the effect of the source of sugar employed (glucose or sucrose) were compared. The medium culture was prepared based on Fang et al. (2006), then modified to avoid precipitation of metals. In each 250 mL bottle, 2 mL of metals solution, 2 mL of yeast solution, 2 mL of phosphates solution, 2 mL of calcium solution ($CaCl_2$) and 0.5 g of ammonium were added. Glucose and sucrose were added at a concentration of 50 g/L and 25 g/L, respectively. These concentrations were chosen to get the same number of monosaccharide units, since sucrose splits into two sugars (glucose and fructose). This keeps the total sugar units equal for the reactions.

In the second experiment campaign, eight configurations were tested to explore: source and content of sugar employed (the commercial sugars among glucose and sucrose resulting most performing from experiment 1 and JWW) and the H_2 yield from the DF of FVW, employed as substrate. The mixture of FVW and JWW was tested in a ratio of 19:1 (95/5 %, marked with A) and 5.67:1 (85/15 %, marked with B), based on VS. These quantities were chosen to decrease as much as possible the utilization of clean water in order to have total solids at 6 %. Since JWW contained 7.7 g/L of glucose, sucrose was added to supply the system with the same amount of monosaccharides provided by JWW. The inoculum was thermally pretreated for each configuration. The ratio between substrate and inoculum was maintained equal to 1:1 based on VS.

In the third experiment campaign, DF was tested on a mixture of FVW and JWW and TSAD was performed. Four configurations were tested: application or not of thermal treatment on FVW and substrate-to-inoculum ratio (S:I) either 2 or 1. Thermal pretreatment was applied to FVW during the first stage of TSAD experiments to test its efficiency in improving the biodegradability of biomasses. A temperature of 60 °C was applied for 30 min as inoculum pretreatment to minimize the energy consumption of the process. FVW was heated in the oven covered by aluminum foil to avoid significant water evaporation from the samples. The ratio between FVW and JWW was chosen depending on the configuration which achieved the highest H_2 in the previous experiment. The second stage of the process, the AD, was performed on DF digestate which acted as a substrate. S:I was maintained at 1 and the inoculum was not pretreated. A summary of configurations characteristics is presented in Table 2. Single-stage AD was performed on the two configurations from the third experiment that showed the highest H_2 yields: one with a substrate-to-inoculum (S:I) ratio of 2:1 and another with a ratio of 1:1.

2.4. Analytical methods and data elaboration

Inoculum, FVW and JWW were characterized in terms of total solids (TS), volatile solids (VS), and elemental analysis (CHNS, O as difference). TS and VS were measured according to (EPA, 2001).

Elemental composition was determined on dry basis through an elemental analyzer (Elementar vario Macro Cube) where solid dried samples of 20 mg were investigated to obtain carbon (C), hydrogen (H), nitrogen (N), sulfur (S) and oxygen (O) percentages. For quantifying sugars content in JWW, HPLC (Prominence Shimadzu, Kyoto, Japan) was employed. It was equipped with a refractive index detector (RID-10A Shimadzu, Kyoto, Japan) and a 300 mm x 7.8 mm ROA-Organic Acid H^+ (8 %) column (Phenomenex, Torrance, USA).

Biogas composition was evaluated through Micro-GC (SRA) equipped with Molsieve 5A (using argon as carrier) and PoraPlot U (using helium as carrier) columns, along with a TCD detector. The composition was analyzed daily along with the biogas volume produced by emptying each gas bag with a 60 mL syringe. For quantifying VFAs, HPLC (model

Table 2
Configurations tested across three experiments: (i) first, varying inoculum thermal pretreatment, culture medium addition, and sugar source; (ii) second, varying sugar source and adding FVW with A = FVW:JWW 19:1 (VS basis) and B = FVW:JWW 5.67:1 (VS basis); (iii) third, varying FVW thermal pretreatment and the S:I ratio.

FIRST EXPERIMENT			
Configuration	**Inoculum Thermal Pretreatment**	**Sugar source**	**Culture medium**
G	NO	Glucose	NO
S	NO	Sucrose	NO
G_M	NO	Glucose	YES
S_M	NO	Sucrose	YES
G_T	YES	Glucose	NO
S_T	YES	Sucrose	NO
G_M_T	YES	Glucose	YES
S_M_T	YES	Sucrose	YES
SECOND EXPERIMENT			
Configuration	**Sugar source**		**FVW**
SUCR_A	Sucrose (A)		NO
SUCR_B	Sucrose (B)		NO
JWW_A	Jam wastewater (A)		NO
JWW_B	Jam wastewater (B)		NO
SUCR_FVW_A	Sucrose (A)		YES
SUCR_FVW_B	Sucrose (B)		YES
JWW_FVW_A	Jam wastewater (A)		YES
JWW_FVW_B	Jam wastewater (B)		YES
THIRD EXPERIMENT			
Configuration	**FVW thermal pretreatment**		**S:I**
JWW_FVW_1:1	NO		1–1
JWW_FVW_2:1	NO		2–1
JWW_FVW_T_1:1	YES		1–1
JWW_FVW_T_2:1	YES		2–1

LC40DXR, Shimadzu, Kyoto, Japan) was employed with a KINETEX 5 μm EVO C18 column, measuring 150 x 4.6 mm (Phenomenex, Torrance, USA).

The experimental data of biogas productions were subjected to one-way ANOVA to compare the mean results of process conditions on DF and TSAD performances. After the ANOVA, Duncan's post-hoc test ($p < 0.05$) was performed.

3. Results and discussion

3.1. Characterization of inoculum and substrates

Inoculum and substrates were characterized for TS, VS, and elemental composition (CHNS analyzer), with results shown in Table 1. TS of inoculum and FVW were similar, while JWW, being highly diluted, had < 1 % TS. Inoculum showed lower VS due to higher inorganic content, consistent with its origin as digestate from agricultural waste while the organic fraction was indicated by the VS/TS ratio (Niya et al., 2023). FVW and JWW, in contrast, had high VS, reflecting their biodegradable organic composition (Martínez-Mendoza et al., 2023). While literature reports a wide variability in TS and VS/TS values of FVW, the average TS content tends to be slightly higher than that observed in this study, typically around 11 % (Magama et al., 2021; Scotto di Perta et al., 2022; Soltan et al., 2024). However, the VS/TS ratio in our samples aligns with the findings of de Quadros et al. (2023), indicating a high content of biodegradable material, making these substrates suitable for biological conversion processes. For elemental composition, the combined use of FVW and JWW yielded a carbon-to-nitrogen (C/N) ratio of ~ 15, within the optimal range (15–30) for microbial activity and eliminating the need for supplementation (Mazzanti et al., 2025).

3.2. Role of the inoculum in dark fermentation

This experimental campaign investigated the role of the mesophilic digestate of CAS used as inoculum in the DF of commercial and pure sugars. The paragraph follows this structure: a brief description of the experimental configuration, an explanation of the related figure and a comparison of our results with those reported in the literature. The study examined and compared the use of thermally pre-treated CAS inoculum (to inhibit methanogenic consortia), the presence or absence of a culture medium, and the impact of the type of sugar used (glucose or sucrose). The study investigated eight configurations. A detailed summary of them is provided in Table 2.

Fig. 1A presents the biogas production (expressed as mean plus standard deviation) over nine days for DF using glucose or sucrose as sugar source. There is no lag phase since biogas production commenced from day 1 in all configurations, demonstrating the effective activation of the inoculum in degrading the respective sugar. However, a stationary phase was observed in most configurations beginning around day 5.

A significant difference in DF performance was observed between configurations fed with glucose (G, G_M, G_T, G_M_T) and those fed with sucrose (S, S_M, S_T, S_M_T). Systems supplied with glucose exhibited significantly lower biogas yields, reaching a maximum of 106 N mL/g_{VS} for G_M configuration, approximately one third the biogas production achieved in sucrose-fed setups. A similar trend was observed for H_2 in Fig. 1B, with sucrose-fed systems outperforming their glucose-fed counterparts, achieving higher yields of both biogas and H_2. Glucose-fed setups experienced a marked reduction in both biogas and H_2 production after the first day, which correlated with a significant drop in pH, visible in Fig. 1D. Although the initial pH was approximately 8 due to the inherent alkalinity of the inoculum, DF process probably led to the production VFAs, resulting in a pH decline across all configurations. In

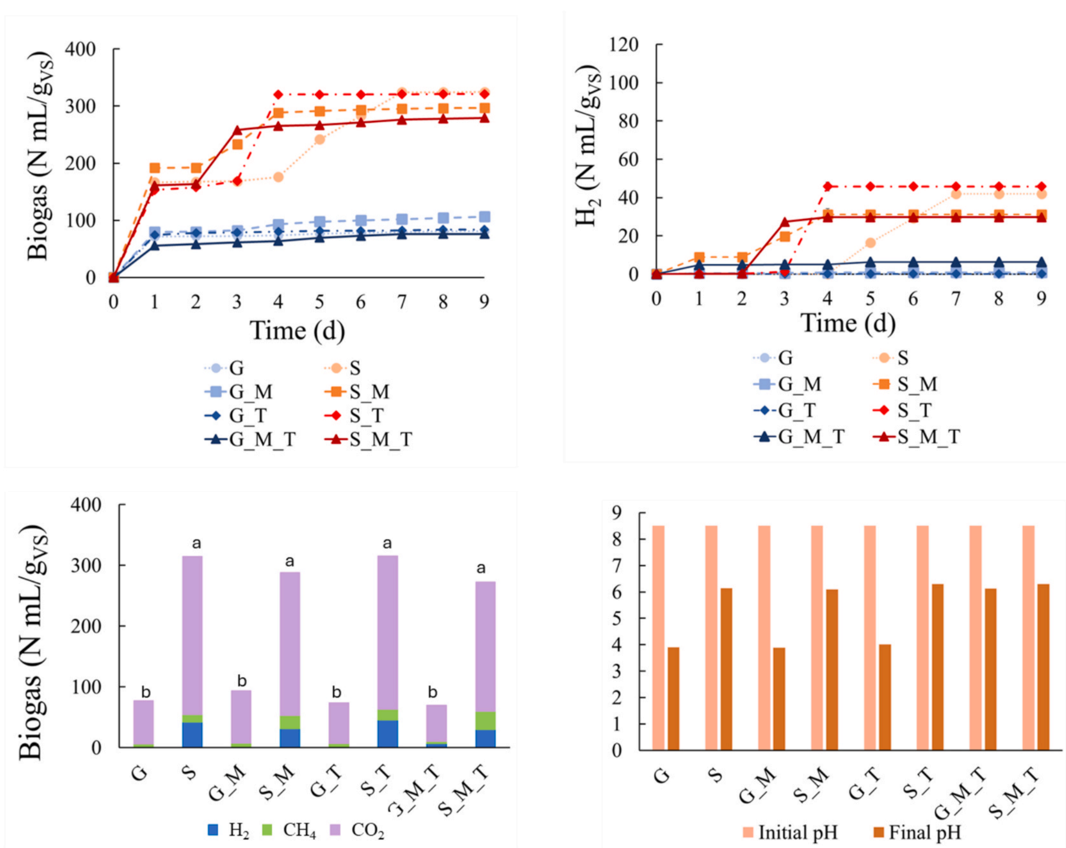

Fig. 1. Results from the first experiment: A) biogas yield, B) H_2 yield, C) composition of total biogas produced during dark fermentation considering H_2, CH_4 and CO_2, D) pH value at day 0 and day 9.

setups using sucrose as the substrate, the pH drop was more moderate, with final values around 6, which is an optimal range for DF activity. In contrast, configurations G, G_M, and G_T exhibited a final pH of approximately 4, a level commonly associated with microbial inhibition and reduced process efficiency (Elbeshbishy et al., 2017). VFAs are toxic to cells because, in their undissociated form, they can cross the cell membrane and disrupt cellular function, potentially leading to the collapse of the microbial community (Chen et al., 2021b). Moreover, a decrease in pH can alter microbial metabolism and suppress H_2 generation (Chen et al., 2021b). Statistical analysis revealed significant differences in the performance of glucose- and sucrose-fed configurations. Specifically, sucrose-fed reactors showed significantly higher performance, highlighting the inoculum's ability to effectively hydrolyze disaccharides and convert them into H_2 and CO_2.

Regarding the effect of thermal pretreatment, no substantial differences in biogas production were observed between untreated and thermally treated inoculum. In sucrose-fed configurations without a culture medium (S and S_T), the treated and untreated inoculum produced 325 and 320 N mL/g_{VS} of biogas, respectively. However, thermal pretreatment did accelerate the onset of maximum H_2 production. Specifically, in thermally treated setups, maximum H_2 production was achieved on day 4, reaching a maximum yield of 45.8 N mL/g_{VS} for S_T, while in untreated inoculum, H_2 production was delayed until day 7, ultimately reaching 42.1 N mL/g_{VS}. Among the glucose-fed setups, only G_M_T produced H_2 (6.5 N mL/g_{VS}), though the yield remained considerably lower compared to that of sucrose-fed configurations. Although final H_2 yields were similar, thermal pretreatment clearly facilitated a faster process startup which is an important advantage for the potential scale-up of DF processes.

Concerning the contribution of the culture medium, its addition enhanced biogas and H_2 production in glucose-fed setups, but the improvements were limited due to the strong inhibitory effects observed after day 1. In sucrose-fed configurations, the addition of a culture medium improved the rate of biogas and H_2 production. When the culture medium was present, H_2 production became visible from day 3, compared to day 4 in setups without the medium. However, the maximum H_2 yield (45.8 N mL/g_{VS}) was achieved in the sucrose-fed configuration without the culture medium (S_T). This finding suggests that mixed cultures are well-suited for dark fermentation due to their resilience and ability to withstand harsh conditions and a culture medium is not strictly necessary (Mohanakrishna and Pengadeth, 2024).

Fig. 1C illustrates the composition of biogas, including H_2, CH_4 and CO_2. In DF processes, the H_2 content in biogas typically ranges between 10 % and 60 %, depending on factors such as temperature and substrate composition (Ghimire et al., 2015). In this study, the H_2 percentage in biogas remained below 15 % in each configuration, reaching a maximum of 14.5 % in S_T. This result was primarily attributed to the premature inhibition of the process, likely caused by the decrease in pH due to the possible accumulation of VFAs alongside biogas production (Chen et al., 2021b). This effect was particularly visible in glucose-fed configurations, where biogas and H_2 production sharply declined after day 1. In all configurations the main component of biogas is CO_2, ranging between 78–92 %. The bars also show a portion corresponding to CH_4 production, ranging from 4 % to 11 %, likely due to the survival of some methanogens after thermal pretreatment. H_2 yields in this study can be compared to those reported by Wang and Wan (2008), who investigated the effects of five different pretreatment methods on digested sludge: thermal (100 °C for 15 min), acid (HCl), alkaline (NaOH), aeration, and chemical (chloroform). All pretreatments enhanced H_2 production from glucose compared to the untreated control, with yields ranging from 85 to 220 mL/g_{VS}. Among these, thermal pretreatment yielded the highest H_2 production, outperforming the results observed in the present study.

Similarly, De Amorim et al. (2012) utilized an anaerobic fluidized bed reactor to produce H_2 from thermally pretreated sludge (90 °C for 10 min), derived from swine wastewater effluent. They achieved a maximum H_2 yield of approximately 130 mL/g_{VS} from glucose, using a hydraulic retention time (HRT) of 2 h and an initial glucose concentration of 2 g/L. The study also found that increasing HRT and glucose concentration led to decreased H_2 production.

Hu and Chen (2007) examined three pretreatment strategies: acidic (HCl), thermal (boiling water for 10–30 min), and chemical (chloroform). They were applied to two inoculum types: anaerobic sewage sludge and methanogenic granules. The culture medium contained 18.75 g/L of glucose. Acid pretreatment had little positive effect on H_2 production for either inoculum. However, chloroform addition at concentrations below 1 % significantly enhanced H_2 production in methanogenic granules, reaching a maximum of 135 mL/g_{VS} at 0.05 % concentration. Thermal pretreatment showed similar benefits, particularly when the granules were boiled for 30 min, resulting in a yield of 118 mL/g_{VS}.

Overall, this experiment demonstrated the viability of CAS inoculum in DF by effectively degrading both monosaccharides, such as glucose, and more complex sugars, such as sucrose. The impact of thermal pretreatment was also evaluated, and while its effect on enhancing total biogas and H_2 production was not particularly visible, it was evident that pretreatment influenced the rate of production by facilitating a faster startup of the process. The role of a culture medium was also investigated, revealing that its presence is not essential for improving process yield. This is an important consideration for scaling up DF, as avoiding the use of a culture medium can lead to reduced operational costs. Furthermore, this study underscored the critical role of pH in DF. The rapid production of VFAs led to a significant decline in pH, ultimately hindering the process.

3.3. *Dark fermentation of fruit and vegetable wastes with jam wastewater*

The second experimental campaign evaluated the technical feasibility of using alternative sugar sources for H_2 production through DF. The paragraph follows this structure: a brief description of the experimental configuration, an explanation of the related figure and a comparison of our results with those reported in the literature. To reduce reliance on commercial sugars, which could be cost-prohibitive at larger scales, sugar-rich biomass wastes were considered potential alternatives. JWW, generated during the cleaning of equipment in a blueberry jam production facility, could be an option. This wastewater contains a monosaccharide concentration of approximately 7.7 g/L. In this study, the performance of JWW as a sugar source for DF was compared to that of commercial sucrose. Both sugar sources were also used in the DF of real biomass waste, specifically FVW, to assess their biocompatibility and effectiveness in H_2 production.

The experiment was conducted considering eight configurations, with half utilizing sucrose as the sugar source (since it was considered the most suitable sugar source according to the main findings of paragraph 3.2) and the other half employing JWW. A detailed summary of them is provided in Table 2.

Fig. 2A and B illustrate biogas and H_2 production across the configurations over a 7-day period. In the reactors containing only sugar sources (SUCR_A, SUCR_B, JWW_A, and JWW_B), the trends in H_2 and biogas production differed noticeably. These configurations produced very low levels of H_2, with yields close to 0 mL/g_{VS}, indicating that the systems failed to initiate effective H_2 fermentation. In contrast, biogas production was relatively high, likely due to CO_2 released from carbon degradation and the presence of methane. No clear difference was observed regarding the use of sucrose or JWW as the sugar source. The low H_2 output may be attributed to the diluted concentration of sugars and the limited availability of nutrients essential for microbial activity and system startup.

On the other hand, when sugar sources were used together with FVW, H_2 production improved across all other configurations. The highest H_2 yields were achieved in JWW_FVW_A and JWW_FVW_B, reaching 67.1 N mL/g_{VS}. These values were higher than those obtained

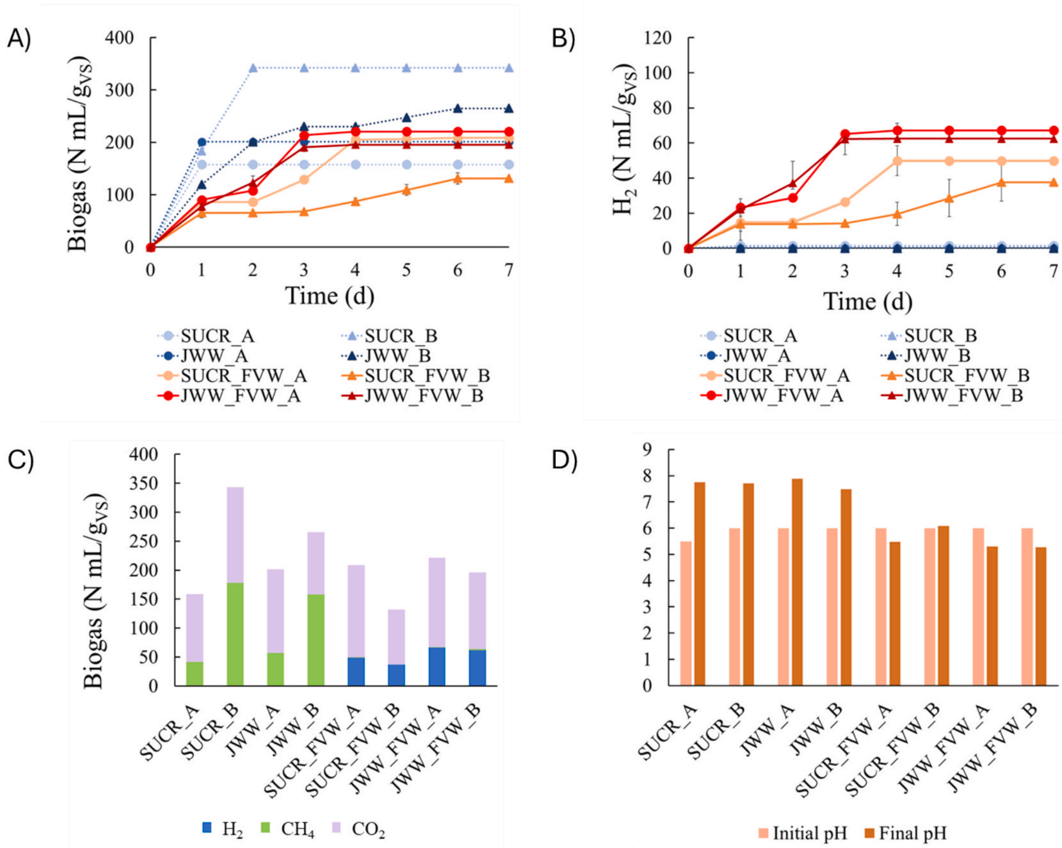

Fig. 2. Results from the second experiment: A) biogas yield, B) H_2 yield, C) composition of total biogas produced during dark fermentation considering H_2, CH_4 and CO_2, D) pH value at day 0 and day 7.

in SUCR_FVW_A and SUCR_FVW_B, which yielded 50 and 37.6 N mL/g$_{VS}$, respectively. These findings suggest that JWW helped increase H_2 production at both tested concentrations, likely due to its content of easily fermentable sugars. The presence of JWW also appeared to enhance the H_2 production rate. Most setups reached a stationary phase by day 4, which is consistent with commonly reported HRT for DF (de Menezes et al., 2024; Scotto di Perta et al., 2022). No significant differences were revealed in biogas production since it remained similar between the setups using sucrose and those using JWW. This suggests that JWW can effectively replace commercial sugars sources in DF, as its performance is comparable to that of sucrose.

Fig. 2C depicts the composition of biogas for all the configurations. H_2 only appears in the configurations with FVW and its maximum concentration in biogas reached 32 % for JWW_FVW_B, 30 % in JWW_FVW_A, 24 % in SUCR_FVW_A and 29 % in SUCR_FVW_B. In these configurations, CO_2 percentage in biogas resulted lower than previous experiment ranging between 48–76 % while CH_4 content was nearly negligible. However, CH_4 was present when only the sugar source was added to the reactors over 25 %. It is not clear why thermal pretreatment was less effective in some cases while it enhanced H_2-producing microbial activity in others. It is possible that when only the sugar source was present in the reactor, the carbohydrate supply was insufficient to support all microbial groups. This may have allowed the surviving methanogens, even though weakened by thermal pretreatment, to dominate and outcompete the H_2-producers.

The initial pH of all configurations (Fig. 2D) was adjusted to between 5.5 and 6, the optimal range for DF. Following the process, SUCR_A, SUCR_B, JWW_A, and JWW_B exhibited elevated final pH values between 7–8, consistent with their low H_2 and likely low VFAs production. In contrast, the pH in SUCR_FVW_A, SUCR_FVW_B, JWW_FVW_A, and JWW_FVW_B remained relatively stable, ranging from 5 to 6. This stability aligns with higher H_2 yields observed in these reactors, likely supported by favorable pH conditions for microbial activity. The results of this study can be compared to those reported by Martínez-Mendoza et al. (2022), who investigated H_2 production from FVW using 0.7 L reactors operated at 37 °C. Their experiment employed an inoculum that underwent thermal pretreatment at 90 °C for 20 min, resulting in a H_2 yield of 73.2 mL/g$_{VS}$. This slightly higher yield, relative to the present study, may be attributed to the more intense heat shock applied to the inoculum. Additionally, the use of pH control through the addition of 6 M NaOH to maintain a neutral pH of 7, may have contributed to improved system performance.

Even higher H_2 production was reported by Magama et al. (2021), who also fermented FVW in 0.4 L reactors at 35 °C. In their case, the inoculum was pretreated with H_2SO_4 to reach a pH of 4 and maintained under these conditions for 24 h. This approach resulted in an H_2 yield of 140 N mL/g$_{VS}$, suggesting that acid pretreatment can be highly effective in enhancing microbial activity and H_2 output.

Scotto di Perta et al. (2022) applied thermal pretreatment by heating the inoculum to 105 °C for several hours and carried out DF in 0.25 L reactors at 30–34 °C. This setup achieved a H_2 yield of 193 N mL/g$_{VS}$, further confirming the effectiveness of thermal pretreatment in optimizing H_2 production from FVW under mesophilic conditions. Overall, this experiment successfully demonstrated the capability of CAS inoculum in converting real waste materials into H_2-rich biogas through DF. However, despite the positive outcomes, both H_2 yield, and concentration were not as high as some values reported in the literature, indicating the necessity for process optimization. Future improvements could focus on refining operational conditions, such as pretreatment strategies and pH regulation, to enhance microbial activity and overall process efficiency. Nonetheless, these results provide a promising foundation for considering the implementation of a TSAD system for

FVW and JWW, which could further optimize biomass utilization and improve energy recovery.

3.4. *Two-stage anaerobic digestion of fruit and vegetable waste and jam wastewater: Dark fermentation*

This experimental campaign evaluated the feasibility of integrating DF and AD in a sequential process, specifically applying a TSAD process to FVW and JWW. The paragraph follows this structure: a brief description of the experimental configuration, an explanation of the related figure with the comparison of our results with those reported in the literature.

Four different configurations were tested in the first stage of TSAD, with variations in the S:I ratio and the application of thermal pretreatment to FVW. The ratio between FVW and JWW was 19:1 on VS basis, associated with the highest H_2 yield in the previous experiment as presented in paragraph 3.3.

In two configurations (JWW_FVW_1:1 and JWW_FVW_T_1:1), the S:I ratio was set to 1, while in the other two, it was increased to 2 (JWW_FVW_2:1 and JWW_FVW_T_2:1), based on VS. The S:I ratio is a critical parameter for maintaining a balanced system (Pan et al., 2008). An insufficient substrate supply can inhibit microbial activity and promote the development of alternative metabolic pathways that are not favorable for optimal energy recovery. Conversely, an excess substrate may lead to excessive sludge growth at the expense of valuable product formation (Cappai et al., 2018). Additionally, thermal pretreatment was applied to FVW at 60 °C for 30 min in two of the configurations (JWW_FVW_T_1:1 and JWW_FVW_T_2:1) to assess its effectiveness in enhancing microbial access to available carbon. Details of the configurations are provided in Table 2.

The effect of varying S:I can be evaluated through the biogas and H_2 yield presented in Fig. 3A. Biogas production commenced from day 1 in all configurations; however, the trends varied significantly among them. The two configurations with an S:I ratio of 2 (JWW_FVW_2:1 and JWW_FVW_T_2:1), exhibited lower biogas production compared to other configurations, and they entered a stationary phase starting on day 1, which persisted throughout the DF period. In contrast, the configurations with an S:I ratio of 1 demonstrated substantially higher biogas yields. The highest production was observed in JWW_FVW_T_1:1, reaching a maximum of 261 N mL/g_{VS}, whereas JWW_FVW_1:1 produced a maximum of 179 N mL/g_{VS}.

While biogas was produced from day 1 in all cases, H_2 production followed a different pattern. In configurations with an S:I ratio of 2, H_2 production was negligible. JWW_FVW_1:1 presented a H_2 yield of 34.3 N mL/g_{VS}, and the process entered a stationary phase after just one day, preventing further substrate conversion in H_2. The highest H_2 yield recorded was 99.5 N mL/g_{VS} for JWW_FVW_T_1:1. The lower efficiency of DF in the high-S:I configurations could be attributed to excessive organic loading, which likely overwhelmed the microbial community and led to system inhibition, resulting in comparable outcomes in both cases. Conversely, the S:I ratio of 1 appeared to provide a more manageable organic load, allowing microorganisms to efficiently perform DF and achieve higher biogas production.

The statistically significant difference in biogas and H_2 yield between thermally pretreated and untreated substrates can likely be explained by the effectiveness of thermal pretreatment in partially degrading the substrate, thereby increasing the availability of simple sugars for microbial metabolism. This trend was particularly evident in JWW_FVW_T_1:1, where the rate of production remained stable, further corroborating findings from the previous experiment, in which a distinct

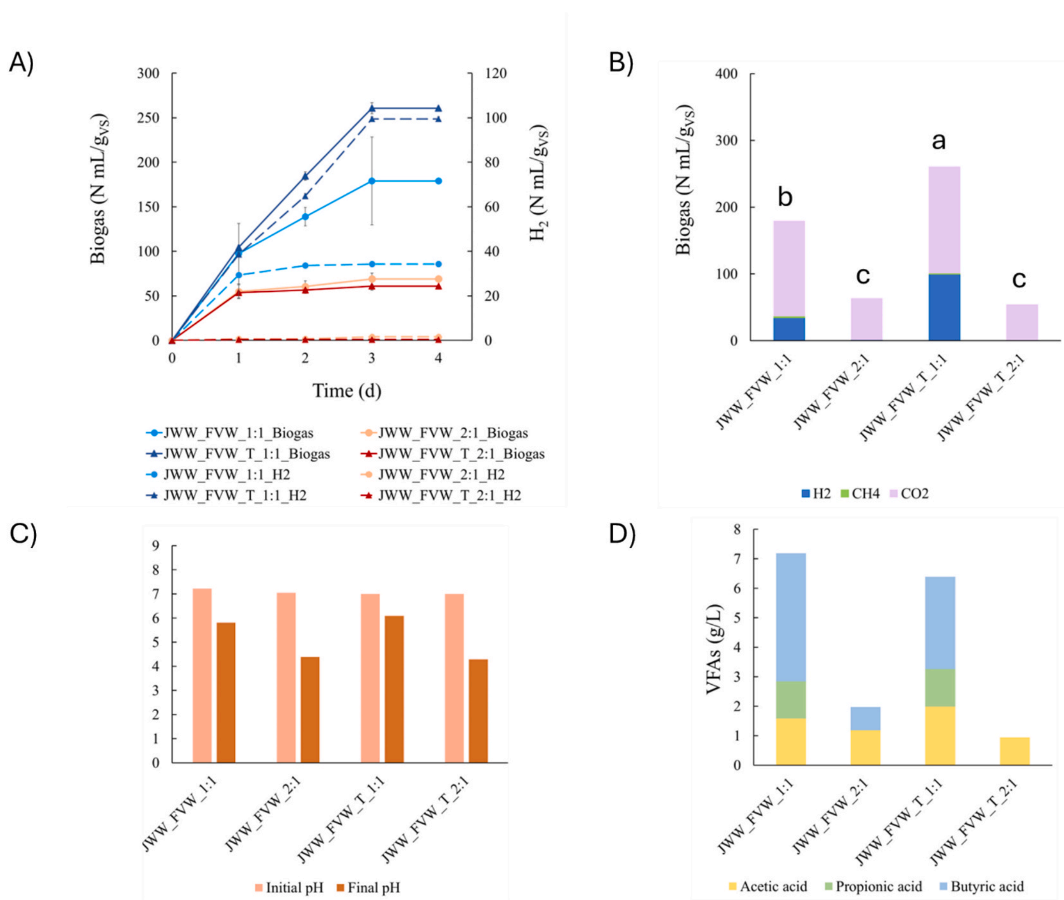

Fig. 3. Results from the first stage of the third experiment: A) biogas (continuous line —) and H_2 yield (dotted line – –), B) composition of total biogas produced during dark fermentation considering H_2, CH_4 and CO_2, C) pH value at day 0 and day 4, D) VFAs content in g/L in the four configurations.

lag phase only became apparent from day 4 of DF. The highest H_2 yield recorded was 99.5 N mL/g_{VS} (JWW_FVW_T_1:1), which was also higher than the maximum yield observed in the previous experiment, highlighting the effectiveness of thermal pretreatment in improving substrate accessibility and enhancing conversion efficiency for H_2 production.

The composition of the produced biogas is presented in Fig. 3B. The H_2 content was higher than the previous experiments (paragraph 3.3), reaching a maximum of 38 % in JWW_FVW_T_1:1. The predominant gas was CO_2 in all the configuration with percentage between 61–99 % while CH_4 content was negligible suggesting the efficacy of the thermal pretreatment.

pH trends are presented in Fig. 3C. The initial pH across all configurations was approximately 7, but it decreased during the process in every case. In JWW_FVW_1:1 and JWW_FVW_T_1:1, the final pH ranged between 5.5 and 6.5 which is an optimal range for DF, and it is correlated with the highest H_2 yields observed. In contrast, JWW_FVW_2:1 and JWW_FVW_T_2:1 experienced a more visible pH drop, reaching values between 4.5 and 5, which likely contributed to the lower H_2 production in these setups.

Numerous studies have investigated the influence of the S:I ratio on H_2 production during DF. Pan et al. (2008) conducted food waste fermentation under mesophilic conditions in 1 L reactors and achieved a H_2 yield of 38.8 mL/g_{VS} at an S:I ratio of 6 (based on VS). Nathao et al. (2013) used a slightly higher ratio of 7.5 for the DF of food waste in 0.5 L reactors at 37 °C, resulting in a yield of 55 mL/g_{VS}. Ghimire et al. (2016), on the other hand, applied a low S:I ratio of 0.5 under thermophilic conditions and obtained 60.6 mL/g_{VS}. Their investigation also highlighted the role of initial pH, identifying 4.5 as optimal for DF which is a result that contrasts with our findings in Section 3.2, where pH values below 5 led to microbial inhibition. Cappai et al. (2018) reported the highest yield among the referenced studies, reaching 90 mL/g_{VS} using a 5 L reactor at 39 °C and an S:I ratio of 7.14, aligning with the conditions applied by Nathao et al. (2013). While several reports suggest that increasing the S:I ratio can enhance H_2 production, the present study observed a different trend, indicating that excessive substrate loading may impair system performance. These findings emphasize the need for careful optimization of the S:I ratio to ensure efficient energy recovery.

The digestate from the first stage of DF was analyzed for VFAs, specifically acetic, propionic, and butyric acids, to assess the progression of the process. The results are presented in Fig. 3D. The distribution of VFAs was highly heterogeneous across the samples. Acetic acid was detected in all reactors (ranging from 1 to 2 g/L), while propionic acid appeared only in half of the cases. The configurations JWW_FVW_1:1 and JWW_FVW_T_1:1 exhibited similar VFAs profiles, with all three acids present in comparable concentrations. JWW_FVW_1:1 had the highest concentration of butyric acid (4.3 g/L), followed by JWW_FVW_T_1:1 (3.1 g/L), making butyric acid the most abundant acid in both cases. Acetic acid concentrations were 1.6 and 2.0 g/L, respectively, while propionic acid was present at 1.3 g/L in both configurations. The total VFAs concentrations were 7.2 g/L for JWW_FVW_1:1 and 6.4 g/L for JWW_FVW_T_1:1. These values exceed the peak of 5.7 g/L reported by Zuo et al. (2014) from the fermentation of vegetable waste but remain below the inhibitory threshold of 8 g/L, as indicated by Cremonez et al. (2021).

The presence of all three acids likely results from the availability of readily fermentable compounds in FVW, which are metabolized through acetyl-CoA and lactate pathways (Ungerfeld, 2020). The predominance of acetic and butyric acids aligns with findings from Dinh and Fujiwara (2023), who reported a VFAs production of 370 mg_{VFAs}/g_{VS} from FVW fermentation which is comparable to the 367 and 326 mg_{VFAs}/g_{VS} observed in JWW_FVW_1:1 and JWW_FVW_T_1:1, respectively.

In contrast, the configurations JWW_FVW_2:1 and JWW_FVW_T_2:1 produced lower VFAs concentrations, with total contents of 2.0 and 0.9 g/L, respectively. While both acetic and butyric acids were detected in JWW_FVW_2:1 (1.2 and 0.7 g/L), only acetic acid was observed in JWW_FVW_T_2:1. The substantially higher VFAs production in the 1:1 S:I ratio configurations can be correlated with better system performance and higher H_2 yields. Conversely, the limited acid production in the 2:1 S:I configurations likely reflect system failure or suboptimal metabolic activity.

The results of this experiment confirm the viability of using a mixture of FVW as a substrate for DF and combining it with JWW as a sugar source. The utilization of a S:1 = 1 on VS basis and a thermal pretreatment of FVW can further enhance the yield of the process, supporting the potential implementation of a TSAD process to further exploit the energy potential of the remaining biomass.

3.5. Two-stage anaerobic digestion of fruit and vegetable waste and jam wastewater: Anaerobic digestion

The second stage of the process (AD) utilized the digestate from the DF stage as the primary substrate. The paragraph follows this structure: a brief description of the experimental configuration, an explanation of the related figure and a comparison of our results with those reported in the literature.

This digestate was combined with non-pretreated CAS inoculum at a 1:1 ratio while maintaining a TS content of 6 %.

The impact of the S:I ratio in the second stage can be evaluated through biogas and CH_4 production results over 22 days, presented in Fig. 4A and B. All configurations exhibited biogas production even at different rates. JWW_FVW_1:1 and JWW_FVW_T_1:1 exhibited a slower initial biogas production, with negligible gas output during this period. However, between days 7 and 11, they began producing biogas at a higher rate than the other two configurations. In the final phase of the experiment, these two configurations experienced a gradual deceleration in biogas production, reaching a plateau around day 21, with a maximum biogas yield of 207 N mL/g_{VS}. CH_4 production remained relatively low across all four configurations during the first 5 days. JWW_FVW_1:1 and JWW_FVW_T_1:1 presented an increase in CH_4 production rate from day 6 to day 11. From day 12, the production rate decelerated, reaching a maximum CH_4 yield of 155 and 146 N mL/g_{VS}, respectively.

JWW_FVW_2:1 and JWW_FVW_T_2:1 exhibited a more rapid start-up phase within the first 6 days in biogas production compared to JWW_FVW_1:1 and JWW_FVW_T_1:1. Configurations with 2:1 ratio presented a continuous increase in biogas production, achieving maximum yields of 279 and 297 N mL/g_{VS}, respectively. These two configurations did not experience a distinct stationary phase, instead maintaining a steady upward trajectory in biogas production throughout the entire experimental period.

Regarding CH_4 yield, JWW_FVW_2:1 and JWW_FVW_T_2:1 exhibited a delayed production phase during the first 7 days. From day 8, an increase in CH_4 production rate was detectable. After day 13, a slowdown in production was observed, while no distinct stationary phase throughout the 22-day period was visible. The highest CH_4 yield (184 N mL/g_{VS}) was achieved by both JWW_FVW_2:1 and JWW_FVW_T_2:1.

Thermal pretreatment of the substrate led to the highest overall biogas yield in the JWW_FVW_T_2:1 configuration. However, statistical analysis showed no significant differences in biogas production during the second stage of TSAD. Although thermal treatment enhanced hydrogen production in the DF, its impact on biogas and CH_4 generation in the subsequent AD phase was limited, indicating that the effect of pretreatment may be specific to the first stage. Similarly, variations in S:I ratio did not significantly influence biogas output in the AD stage, as no statistical differences were observed among the tested configurations.

Fig. 4C shows the biogas composition of all configurations. The main component in all cases is CH_4 which reaches 81 % in JWW_FVW_1:1, 65 % in JWW_FVW_T_2:1, 73 % in JWW_FVW_T_1:1 and 61 % in JWW_FVW_T_2:1. CO_2 levels were lower with percentage ranging between 19 and 37 %. Interestingly, even if JWW_FVW_1:1 and JWW_FVW_T_1:1 presented a lower CH_4 yield, the relative CH_4

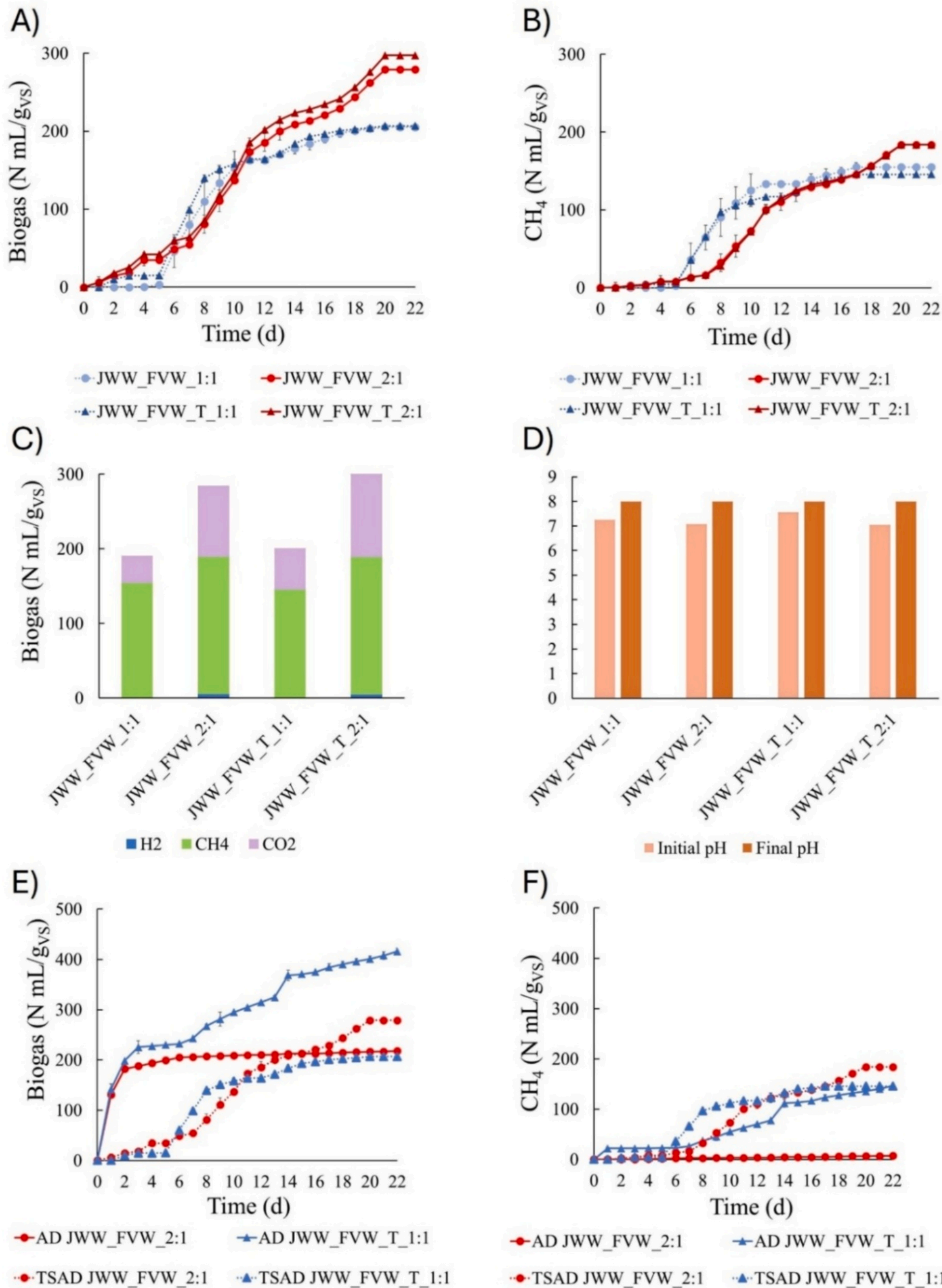

Fig. 4. Results from the second stage of the third experiment: A) biogas yield, B) CH_4 yield, C) composition of total biogas produced during anaerobic digestion considering H_2, CH_4 and CO_2, D) pH value at day 0 and day 22, E) biogas yield between the application of TSAD or AD to JWW_FVW_2:1 and JWW_FVW_T_1:1, F) CH_4 yield between the application of TSAD or AD to JWW_FVW_2:1 and JWW_FVW_T_1:1.

percentages were higher compared to JWW_FVW_2:1 and JWW_FVW_T_2:1.

The pH trends of the four configurations are shown in Fig. 4D. Both initial and final pH values ranged between 7 and 8, which is considered optimal for AD. These results suggest that pH was not a limiting factor for the suboptimal CH_4 yields observed.

The performance of the TSAD system in this study can be compared with previous research. Dinh and Fujiwara (2023) investigated TSAD of FVW using sequential acidogenic and methanogenic reactors. The first stage operated at 35 °C, while the second was tested under both mesophilic and thermophilic conditions, obtaining a CH_4 yield of 306 mL/g_{VS} and 346 mL/g_{VS}, respectively. These higher values, compared to those reported in the present study, may be attributed to the feeding strategy: only the liquid fraction of the digestate, rich in VFAs, was used in the methanogenic reactor, whereas the full digestate was fed in this work.

Similarly, Dinh et al. (2019) applied TSAD to vegetable waste using a CSTR for DF and an upflow anaerobic sludge blanket (UASB) reactor for methanogenesis at 36 °C. Although little to no H_2 was detected during the first stage (with biogas primarily composed of CO_2), the second stage produced 274 mL/g_{VS} of CH_4. Wu et al. (2016) also reported comparable outcomes, applying TSAD to FVW using a 1.2 L CSTR for DF and a mesophilic 1 L UASB reactor for AD. In this case, H_2 production was again negligible in the first stage, while CH_4 yield reached 244 mL/g_{VS}. Zuo et al. (2014) conducted TSAD using a 3 L CSTR for DF and a 4 L fixed-bed biofilm reactor for methanogenesis, both under mesophilic conditions. Although no H_2 data was provided, the CH_4 yield was approximately 300 mL/g_{VS}.

The results obtained in this study fall between those previously reported and those achieved by Nathao et al. (2013), who applied TSAD to food waste. H_2 production reached 55 mL/g_{VS}, and CH_4 yield was 94.8 mL/g_{VS}, obtained from batch reactors operating at 37 °C. The lower CH_4 production compared to this study could be due to the composition of the substrate, which likely contained a broader range of organic matter, including proteins and fats in addition to carbohydrates. Gómez Camacho et al. (2019) also investigated TSAD using organic market waste. DF and AD were conducted in 2 L and 14 L bioreactors, respectively, both at 35 °C. The H_2 yield was 50 mL/g_{VS}, while CH_4 production reached 179 mL/g_{VS}. Although H_2 yields were slightly lower than those reported in the present study, the CH_4 aligns with the results of this study.

These findings emphasize that the effects of thermal pretreatment and the substrate-to-inoculum ratio are stage-specific within TSAD process. Although the CH_4 yield was satisfactory, it remains lower than that reported in similar studies, indicating that process optimization may be necessary.

3.6. Comparison between single and two-stage anaerobic digestion of fruit and vegetable waste

As previously mentioned in paragraphs 3.4 and 3.5, the implementation of TSAD rather than single stage AD, is expected to enhance CH_4 production. To evaluate this hypothesis within the scope of this study, AD was applied to the configurations JWW_FVW_2:1 and JWW_FVW_T_1:1, following the criteria stated in paragraph 2.3.

Biogas and CH_4 yields are presented in Fig. 4E and F. Among all configurations, AD of JWW_FVW_T_1:1 yielded the highest biogas output at 416 N mL/g_{VS}, significantly outperforming the others, which ranged between 206 and 279 N mL/g_{VS}. However, the trend for CH_4 production was visibly different. The highest CH_4 yield (184 N mL/g_{VS}) was achieved by TSAD JWW_FVW_2:1, which was substantially higher than the corresponding AD configuration, which produced only 7.5 N mL/g_{VS}. This highlights the positive impact of the DF stage in enhancing the overall digestion performance, likely due to the initial hydrolysis and breakdown of complex organic matter. In contrast, applying AD directly to JWW_FVW_2:1 is suboptimal, possibly due to an excessive organic load that exceeded the inoculum metabolic capacity. Both TSAD and AD applied to JWW_FVW_T_1:1 resulted in similar CH_4 yields of approximately 146 N mL/g_{VS}.

Variations in specific methane production rates can be observed and assessed based on the slope of the cumulative yield curves. TSAD configurations showed a clear onset of CH_4 production around days 6 and 8, respectively. Conversely, AD JWW_FVW_2:1 showed an almost flat production curve, while AD JWW_FVW_T_1:1 exhibited a delayed but sharp increase starting at day 14, which was nearly double the stationary phase compared to its TSAD counterpart. These findings indicate that TSAD not only enhances CH_4 yield but also accelerates the production rate, enabling faster reaching of peak yields which is an advantage of considerable relevance for industrial-scale applications where time efficiency is critical. Previous studies also supported the benefits of TSAD for improving CH_4 recovery. Nathao et al. (2013) compared TSAD and AD of food waste in batch reactors at 37 °C and reported an 18 % increase in energy recovery with TSAD. Similarly, Liu et al. (2006) evaluated TSAD and AD of household solid waste under mesophilic conditions and observed a 21 % increase in CH_4 yield from the TSAD process. These studies further reinforce the advantages of TSAD for maximizing energy recovery from biomass waste.

4. Conclusion

The study explored inoculum preparation and operational conditions during dark fermentation. Thermal pretreatment at 60 °C for 30 min promoted H_2-producing microorganisms, allowing the process to proceed without culture medium, supporting its scalability. JWW was successfully subjected to DF, alone and with FVW, with a maximum H_2 yield of 67.1 N mL/g_{VS} from untreated FVW. Applying thermal pretreatment to FVW and a 1:1 S:I ratio enhanced H_2 yield to 99.5 N mL/g_{VS} and VFAs to 7.2 g/L. In the second stage, CH_4 production reached a maximum of 184 N mL/g_{VS} in JWW_FVW_2:1, but no significant difference was found in biogas production from all the configurations. Compared to single-stage AD, TSAD improved CH_4 output. These results confirm JWW as a viable, low-cost alternative to commercial sugars in DF and TSAD, supporting circular economy principles. Further research is needed to optimize operational parameters and maximize energy recovery.

CRediT authorship contribution statement

Gaia Mazzanti: Writing – original draft, Formal analysis, Data curation. **Francesca Demichelis:** Supervision, Methodology, Conceptualization. **Debora Fino:** Supervision, Conceptualization. **Tonia Tommasi:** Supervision, Methodology, 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.

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Data availability

Data will be made available on request.

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