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Research Article

Coupling NaDES-Based Polyphenol Extraction and Anaerobic Fermentation in Grape Pomace Biorefineries: Experimental Evidence and Stoichiometric Assessment of Residual Solvent Effects

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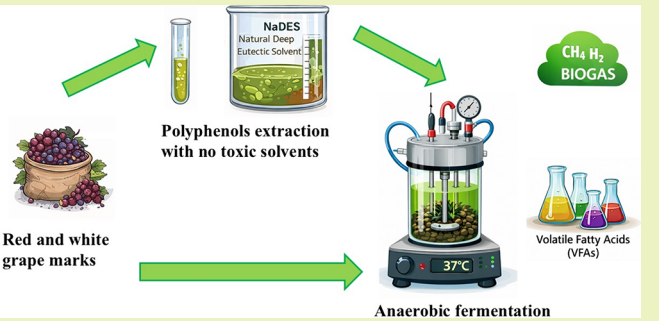
ABSTRACT: Grape pomace (GP) is a promising feedstock for integrated biorefineries that combine the recovery of high-value compounds with biological conversion processes. In this study, polyphenol extraction using Natural Deep Eutectic Solvents (NaDES) was coupled with anaerobic fermentation of white and Red-GP operated at two hydraulic retention times (10 and 20 days) at 37 ± 1 °C. The selected NaDES comprised glucose–citric acid–water (GCW) or glucose–lactic acid–water (GLW). Polyphenol extraction was substrate-dependent: NaDES recovered approximately 3.0–6.8 mg GAE g^{-1} from white GP, below MeOH/HCl (11.2 mg GAE g^{-1}), whereas 3.6–4.6 mg GAE g^{-1} were recovered from Red-GP, comparable to MeOH/HCl (4.6 mg GAE g^{-1}). Untreated substrates exhibited distinct fermentation behaviors: white GP achieved higher total biogas yields (180–250 L $kgVS^{-1}$), whereas Red-GP produced H_2-rich biogas at an HRT of 10 days (approximately 39% H_2) and CH_4-rich biogas at an HRT of 20 days (approximately 80% CH_4). In contrast, NaDES-pretreated GP showed a drastic reduction in biogas production (3–17 L $kgVS^{-1}$), with the biogas composed almost exclusively of CO_2. A stoichiometric model revealed that NaDES carry-over accounted for a substantial solvent-derived fraction of the influent COD, corresponding to up to 0.93 g COD g^{-1} GP for glucose–citric acid systems and 0.34 g COD g^{-1} GP for glucose–lactic acid systems. This readily biodegradable carbon stream, together with substrate modification induced by extraction, reshaped microbial pathways, promoting acidogenesis and the accumulation of soluble intermediates. Volatile fatty acid (VFA) profiles were strongly influenced by NaDES composition: glucose–citric acid systems favored acetate-dominated patterns, whereas glucose–lactic acid systems enabled the formation of more reduced products and caproate (up to ~8% w/w) at longer retention times. However, NaDES pretreatment did not enhance overall caproate production compared to untreated GP. Overall, the results demonstrate that NaDES cannot be regarded as biologically inert extraction media. Their carry-over introduces a composition-dependent carbon input that markedly alters fermentation performance, highlighting the need for solvent management strategies when integrating NaDES extraction with anaerobic bioconversion in GP biorefineries.



KEYWORDS: *grape pomace, polyphenols, anaerobic fermentation, NaDES, volatile fatty acids, chain elongation, biorefinery*

1. INTRODUCTION

The global wine industry generates large quantities of organic residues, among which grape pomace (GP) represents the most abundant solid by-product. According to the International Organization of Vine (OIV), global grape production exceeds 80 million tons per year, with Europe accounting for a substantial share of total output. GP, consisting mainly of grape skins, seeds, and stems remaining after juice extraction, typically represents 10–20% (w/w) of the processed grapes and accounts for up to 60% of total winery solid waste.[1] From a compositional perspective, GP is a heterogeneous lignocellulosic matrix containing cellulose, hemicellulose, lignin, residual sugars, lipids, proteins, and minerals. In addition, GP is particularly rich in polyphenolic compounds, which are only partially transferred from grapes to wine during vinification.[1] The phenolic profile and concentration strongly depend on grape variety, cultivation conditions, and winemaking practices, with red grape pomace generally containing higher amounts of polyphenols than white grape pomace due to prolonged skin contact during fermentation.[2] Several valorization routes for GP have been proposed and implemented at different technological readiness levels.[3]

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Traditional applications include distillation for the production of spirits (e.g., grappa), use as animal feed, and land spreading or composting. However, these routes are often limited by low economic returns, seasonal availability, and potential environmental drawbacks. In recent years, biological conversion pathways have attracted increasing attention, particularly within integrated biorefinery schemes aimed at maximizing resource efficiency and product diversification.[4]

Anaerobic digestion (AD) is a well-established biological technology for the treatment and valorization of organic waste streams. Through a complex microbial consortium operating under oxygen-free conditions, AD converts organic matter into biogas, mainly composed of methane and carbon dioxide, and digestate, which can be used as a biofertilizer. In addition to methane, intermediate products such as hydrogen and volatile fatty acids (VFAs) can be recovered by controlling operational parameters and microbial pathways.[5] Despite its potential, the AD of GP is often challenging. Several studies have reported relatively low or highly variable biomethanation performances compared to other agro-industrial residues. This result is commonly attributed to the lignocellulosic nature of GP, which limits hydrolysis rates, as well as to the presence of potentially inhibitory compounds.[5] Among these, polyphenols have been frequently cited as microbial inhibitors due to their antimicrobial activity, potentially leading to prolonged lag phases, reduced methane yields, and process instability. However, the actual inhibitory effect of polyphenols strongly depends on their concentration, chemical structure, and bioavailability.[1,6] Experimental evidence suggests that inhibition generally occurs only above specific threshold concentrations, which may not always be reached under typical anaerobic digestion conditions.

Biochemical methane potential (BMP) tests on GP typically report methane yields in the order of a few hundred milliliters of CH_4 per gram of volatile solids, with substantial variability between white and red pomace and between untreated and pretreated substrates.[7,8] Operational factors such as hydraulic retention time (HRT), organic loading rate (OLR), and process stability often play a more decisive role than substrate pretreatment alone in determining final yields.[9,10] To overcome the limitations associated with direct AD, the extraction of polyphenols prior to AD has emerged as an appealing option within integrated biorefinery concepts.[9] This approach aims to recover high-value bioactive compounds while potentially improving downstream biological conversion by reducing the concentration of inhibitory substances.

In this context, green extraction technologies based on Natural Deep Eutectic Solvents (NaDES) have recently gained attention as sustainable alternatives to conventional organic solvents. NaDES are typically obtained by combining two or more natural metabolites, such as organic acids, sugars, or amino acids, which interact through hydrogen bonding to form liquids with melting points significantly lower than those of the individual components. This class of solvents is characterized by low volatility, tunable polarity, high thermal stability, and a strong solubilization capacity toward phenolic compounds, making NaDES particularly suitable for application in integrated biorefinery schemes.[11] Despite these advantages, their application within biorefinery concepts remains at an early stage, and the interaction of NaDES with anaerobic processes, as well as their potential contribution to the composition of the downstream influent COD in downstream processes, is still poorly understood. For instance, residual NaDES components entrained in the extracted solid fraction or liquid streams may influence AD in contrasting ways, depending on their concentration, chemical composition, and bioavailability. On one hand, organic acids such as lactic and citric acid may act as easily fermentable substrates or metabolic intermediates, potentially stimulating acidogenesis and VFA production. On the other hand, high local concentrations of NaDES or their strong hydrogen-bonding networks could alter microbial membrane permeability, osmotic balance, or enzyme activity, leading to inhibitory or selective effects on specific functional groups within the anaerobic community.

Against this background, this study investigated the compatibility of NaDES-based polyphenol extraction with the subsequent anaerobic fermentation of white and Red-GP. Untreated and NaDES-extracted substrates were compared at two hydraulic retention times to assess changes in methane, hydrogen, VFA, and MCFA production. Particular attention was given to NaDES carry-over, which introduced a solvent-derived fraction within the fixed influent COD and thereby altered its composition and biodegradability. A stoichiometric model and sensitivity analysis were used to estimate the potential contribution of retained NaDES components and evaluate the effect of different solvent-retention and recovery scenarios. The originality of this work therefore lies in the combined experimental and modeling assessment of an integrated extraction–fermentation process, explicitly considering residual NaDES as an active component of the downstream biological system rather than as an inert extraction medium.

2. MATERIALS AND METHODS

2.1. Substrate Collection and Characterization

White and red grape pomace, Wh-GP and Red-GP, respectively, were collected from winery residues generated after grape pressing and vinification of Garganega and Corvinone. The substrates consisted mainly of grape skins, seeds, and residual stems. Upon collection, the GP samples were stored under refrigerated conditions until further use in order to prevent uncontrolled biological degradation. The substrates were homogenized and characterized to determine their main physicochemical properties. The main chemical and physical features of Wh-GP and Red-GP are reported in Table 1.

Both GPs were characterized by a high organic matter content, with VS/TS ratios of approximately 0.96 and 0.86 for Wh-GP and Red-GP, respectively, and COD values close to 1 kg O_2 $kgTS^{-1}$. Wh-GP showed slightly higher TS, VS, COD, and total polyphenol content than Red-GP, reflecting the different grape varieties and vinification processes from which the two residues originated.

2.2. NaDES Composition and Preparation and Physicochemical Characterization

The NaDES formulations used in this study were selected from previous literature.[11,12] Glucose–citric acid–water systems (GCW, molar ratios 2:1:4, 2:2:4, and 1:2:4) and glucose–lactic acid–water systems (GLW, molar ratios 1:7:6, 1:6:6, and 2:6:6) were prepared following the heating method reported by Dai et al.[13] The components were combined at the selected molar ratios and heated at 60 °C under magnetic stirring until clear and homogeneous liquids were obtained. The pH measurements were performed at room temperature using a pH meter (edge™ HI2020, Hanna Instruments, Italy). Density was measured at 25 °C and atmospheric pressure by means of borosilicate glass pycnometers (BLAUBRAND, Merck KGaA, Germany). Viscosity was assessed using a rotational rheometer (DSR 500 CP4000, Lamy Rheology, France) equipped with a cylindrical probe (MS-DIN11), applying shear rates between 50 and 300 s^{-1}. Electrical conductivity

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Table 1. Main Characterization of White and Red Grape Pomace[a]

	TS (%w/w)	VS (% w/w)	VS/TS (% w/w)	COD (gO_2 $kgTS^{-1}$)	TKN (mgN kg^{-1})	polyphenols contents (mg gallic acid g^{-1})
inoculum	7.46 ± 0.22	4.85 ± 0.29	65.01 ± 0.38	656.95 ± 21.04	5.23 ± 0.64	n.d.
Wh-GP	25.46 ± 1.10	24.43 ± 1.29	96.05 ± 1.01	969.76 ± 22.27	0.62 ± 0.06	11.16 ± 2.98
Red-GP	23.00 ± 3.32	19.70 ± 1.57	86.01 ± 1.69	919.75 ± 55.49	0.53 ± 0.02	4.61 ± 0.21

[a]n.d. = not detected.

was measured using the same edge™ HI2020 instrument fitted with a digital conductivity probe (HI 763100). Molecular interactions among NaDES components were analyzed by FTIR–ATR spectroscopy using a Nicolet 6100 FTIR spectrometer (Thermo Scientific, USA). Spectra were recorded in the range 400–4000 cm^{-1}.

2.3. Substrate Pretreatment

Red-GP and Wh-GP pretreatment was performed according to a modified procedure reported by Sportiello et al.[11] Briefly, 1 g of grape pomace was mixed with 10 mL of NaDES in a Falcon tube and homogenized by vortexing for 1 min. Samples were then shaken on a rotating disk (UniLOPMIX2, LLG-Labware, Germany) at 40 rpm for 30 min, followed by ultrasound-assisted extraction (45 kHz, 50 min; SOLTEC, Italy). After centrifugation (3900 rcf, 10 min), 9 mL of supernatant was collected. All steps were conducted at 25 ± 2 °C under dark conditions. The procedure described above represented a single extraction batch. To obtain the amount of extracted GP required for the semi-continuous fermentation experiments, multiple batches were processed in parallel under identical conditions, maintaining the same GP-to-NaDES ratio and extraction parameters. Following centrifugation, the NaDES-rich supernatants were removed, while the extracted GP fractions were pooled and homogenized before preparation of the fermentation feed. Although most of the NaDES was separated with the supernatant, a residual fraction remained associated with the solid matrix, as quantified by the mass-balance approach described in Section 2.6.

2.4. Anaerobic Fermentation Tests

2.4.1. Experimental Setup and Operating Conditions. Anaerobic fermentation tests were carried out in laboratory-scale reactors operated under mesophilic conditions (T = 37 ± 1°C). The reactors were run in semi-continuous mode and were equipped with gas-tight systems for biogas collection. An anaerobic inoculum was used to initiate the fermentation process, represented by an agricultural digestate (Table 1) from a full-scale plant treating animal manure and corn straw, which was filtered at 500 μm to remove the main solid particles before its adoption for the tests. The inoculum-to-substrate ratio of 1:1 on a VS base was selected to ensure process stability and active microbial metabolism. This ratio referred exclusively to initial reactor inoculation, whereas subsequent operation was defined by the reported HRT, OLR, and daily exchange volumes reported in Table 2. All reactors were operated at a controlled temperature and maintained under strictly anaerobic conditions throughout the experimental period. The pH of the fermentation broth was regularly monitored and manually adjusted to 7.0, when necessary, using 1 M NaOH or 1 M HCl solutions to maintain conditions compatible with anaerobic microbial activity.

2.4.2. Experimental Design and Tested Substrates. A total of 12 semi-continuous anaerobic fermentation tests were conducted to evaluate the effects of grape pomace type, prior NaDES-based polyphenol extraction, NaDES formulation, HRT, and OLR, on biogas, biohydrogen, biomethane, VFA, and caproic acid production. All experiments were performed in 0.5 L glass reactors with a working volume of 250 mL.

Four tests were conducted using untreated Wh-GP and Red-GP, respectively, each operated at HRTs of 10 and 20 days. Eight additional tests were conducted using grape pomace subjected to prior polyphenol extraction (labeled as Extr-GP). Both Wh-GP and Red-GP were extracted using the two selected NaDES formulations, GCW 2:2:4 and GLW 1:7:6, and subsequently fermented at HRTs of 10 and 20 days.

For all experimental conditions, the feed slurry was prepared by diluting the untreated or extracted GP matrix with tap water to a total COD concentration of 50 g COD L^{-1}. For the extracted substrates, this concentration included the COD associated with both the GP and the residual NaDES retained within the treated solid matrix. Therefore, all reactors operated at the same nominal influent COD concentration, irrespective of pretreatment.

At an HRT of 10 days, 25 mL of fermentation broth was withdrawn daily and replaced with an equal volume of fresh feed, corresponding to an OLR of 5.00 g COD L^{-1} d^{-1}. At an HRT of 20 days, the daily

Table 2. Experimental Design and Operating Conditions of the Semi-continuous Anaerobic Fermentation Tests

substrate	NaDES formulation	HRT (d)	OLR (g COD L^{-1} d^{-1})	daily feed/withdrawal (mL d^{-1})	total feed COD (g COD L^{-1})
Wh-GP	–	10	5.00	25.0	50
Wh-GP	–	20	2.50	12.5	50
Red-GP	–	10	5.00	25.0	50
Red-GP	–	20	2.50	12.5	50
Extr-Wh-GP	GCW 2:2:4	10	5.00	25.0	50[a]
Extr-Wh-GP	GCW 2:2:4	20	2.50	12.5	50[a]
Extr-Red-GP	GCW 2:2:4	10	5.00	25.0	50[a]
Extr-Red-GP	GCW 2:2:4	20	2.50	12.5	50[a]
Extr-Wh-GP	GLW 1:7:6	10	5.00	25.0	50[a]
Extr-Wh-GP	GLW 1:7:6	20	2.50	12.5	50[a]
Extr-Red-GP	GLW 1:7:6	10	5.00	25.0	50[a]
Extr-Red-GP	GLW 1:7:6	20	2.50	12.5	50[a]

[a]For extracted GP, the reported total feed COD included both GP-derived organic matter and the residual NaDES retained within the solid matrix. Feed slurries were prepared using tap water as the dilution medium.

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exchange volume was 12.5 mL, corresponding to an OLR of 2.50 g COD L^{-1} d^{-1}. Each experiment was operated for three HRTs, corresponding to 30 days for the HRT 10 d conditions and 60 days for the HRT 20 d conditions. These HRT values were selected to compare a shorter-retention, higher-loading condition with a longer-retention, lower-loading condition while maintaining the influent COD concentration at 50 g COD L^{-1}. The complete experimental design is summarized in Table 2.

The performance of biohydrogen, biomethane VFA, and MCFA production was evaluated by the corresponding substrates conversion yields, referred to the daily inlet VS and COD amount (g_{CODin}) (Eqs 1 and 2). Gas volumes were normalized to standard conditions of 0 °C and 1 atm.

$$Y_{biogas,H_2,CH_4} = \frac{mL_{biogas,H_2,CH_4}/d}{gVSin/d} \quad (1)$$

$$Y_{VFAs} = \frac{(gCOD_{VFAs}/d)}{gCOD_{in}/d} \quad (2)$$

2.5. Analytical Methods

For all experimental runs, biogas production was quantified using the water displacement method. Methane and hydrogen concentrations were determined by gas chromatography using a MicroGC Fusion® Gas Analyzer (INFICON), with argon and helium employed as carrier gases. VFA concentrations were analyzed by ion chromatography using a Dionex Aquion system (Thermo Fisher Scientific, USA) equipped with a Dionex IonPac ICE-AS1 analytical column (4 × 250 mm). According to the column specifications, a 1 mM heptafluorobutyric acid solution was used as the eluent at a flow rate of 0.16 mL min^{-1}. Calibration curves were obtained using serial dilutions of a volatile fatty acid standard mixture (free acid form, 10 mM), supplied by Merck. Medium-chain fatty acids (MCFAs, C7–C12) were analyzed only for the hydraulic retention time that exhibited the highest VFA and caproic acid production. These analyses were performed by an external accredited laboratory in accordance with standardized procedures.[14] Reported methane, hydrogen, VFA, and MCFA values were calculated as mean values from measurements collected during the final two HRTs. Error bars represent the corresponding standard deviations and describe the temporal variability observed within each semi-continuous reactor. Since each operating condition was represented by one independent reactor, repeated measurements within the same reactor were not considered independent biological replicates, and inferential statistical comparisons among conditions were not performed. Total solids (TS), volatile solids (VS), chemical oxygen demand (COD), and total Kjeldahl nitrogen (TKN) were determined following standard methods as described in the literature.[15] Total phenolic content was assessed using the Folin–Ciocalteu assay following Simonato et al.[16] Extracts were reacted with Folin–Ciocalteu reagent and sodium carbonate, and absorbance was measured at 750 nm. Results were expressed as mg gallic acid equivalents per g of dry matter (mg GAE g^{-1} DM) using a gallic acid calibration curve.

2.6. Estimation of COD Associated with Retained NaDES by a Stoichiometric Model

Following the polyphenol extraction step, a fraction of the NaDES remained associated with the Extr-GP due to solvent retention within the solid matrix. Based on mass-balance considerations during the extraction and separation steps, the retained solvent was estimated at approximately 1 mL per g of treated GP. To evaluate the potential contribution of NaDES carry-over to the organic load in the anaerobic reactors, the theoretical COD associated with the retained solvent was calculated. The estimation was based on: (i) the retained NaDES volume, (ii) the measured glucose concentration in the selected NaDES formulations (Table 3), and (iii) the stoichiometric composition of the NaDES mixtures. COD conversion factors of 1.067 gCOD g^{-1} were adopted for glucose and lactic acid, and 0.75 gCOD g^{-1} for citric acid, based on their theoretical oxygen demand.

For the GCW 2:2:4 formulation, assuming a 1:1 molar ratio between glucose and citric acid, the citric acid concentration was estimated from the glucose content by considering their molecular weights. For the GLW 1:7:6 formulation, the lactic acid concentration was estimated analogously based on the molar ratio with glucose.

A stoichiometric model was developed to quantify the theoretical COD introduced into the anaerobic fermentation system through NaDES carry-over. The model considers the retained solvent volume V_{ret}(mL·g^{-1} GP), the concentration of each NaDES component (g·mL^{-1}), and the corresponding COD conversion factors.

For each NaDES formulation, the COD contribution was calculated as:

$$COD_{NaDES} = V_{ret} \cdot \sum_i C_i \cdot f_{COD,i} \quad (3)$$

where C_i is the concentration of component i (glucose, citric acid, lactic acid), and $f_{COD,i}$ is the theoretical COD factor (1.067 gCOD·g^{-1} for glucose and lactic acid; 0.75 gCOD·g^{-1} for citric acid).

For GCW 2:2:4, the citric acid concentration was estimated assuming a 1:1 molar ratio with glucose:

$$C_{cit} = C_{glu} \cdot \frac{MW_{cit}}{MW_{glu}} \quad (4)$$

For GLW 1:7:6, the lactic acid concentration was estimated as:

$$C_{lac} = C_{glu} \cdot \frac{MW_{lac}}{MW_{glu}} \cdot 7 \quad (5)$$

Sensitivity analyses were performed by varying V_{ret} from 0 to 1.5 mL·g^{-1} and simulating solvent recovery efficiencies of 0, 50, and 90%. Additional details of the dataset used for the sensitivity analysis are reported in Supplementary Material S2. The proposed stoichiometric framework should be regarded as a quantitative tool for interpreting the potential magnitude of solvent-derived organic inputs rather than a direct demonstration of carbon partitioning mechanisms. However, it allows assessment of the potential magnitude of the solvent-derived fraction within the influent COD.

Table 3. Characterization of NaDES

NaDES	pH	density (g cm^{-3})	viscosity at 100 s^{-1} (mPa*s)	conductivity (mS cm^{-1})	glucose content (g mL^{-1})
GCW 2:1:4	0.92 ± 0.05	1.27 ± 0.01	17.6 ± 0.45	1.24 ± 0.07	0.49
GCW 2:2:4	0.45 ± 0.04	1.36 ± 0.00	130.2 ± 1.90	0.21 ± 0.00	0.50
GCW 1:2:4	0.55 ± 0.01	1.27 ± 0.00	14.5 ± 0.43	2.11 ± 0.16	0.24
GLW 1:7:6	1.39 ± 0.05	1.07 ± 0.00	2.8 ± 0.34	5.13 ± 0.04	0.07
GLW 1:6:6	1.05 ± 0.02	1.15 ± 0.00	6.2 ± 0.19	1.12 ± 0.01	0.11
GLW 2:6:6	1.36 ± 0.01	1.10 ± 0.00	3.0 ± 0.16	4.04 ± 0.01	0.27

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3. RESULTS AND DISCUSSION

3.1. Characterization of NaDES and Polyphenol Extraction

The NaDES used for the GP pretreatment were first characterized in terms of pH, density, viscosity, and electrical conductivity (Table 3).

Overall, the pH values were very low, in agreement with previous literature reports.[11,17] The low pH values are consistent with the presence and relative proportions of citric or lactic acid in the formulations; however, direct comparison with published values remains limited because few studies have characterized NaDES prepared with identical components and molar ratios. The acidic nature of the systems can be attributed mainly to the presence of organic acids, namely, citric and lactic acid, which dominate the acid–base behavior of the mixtures.

Marked differences were observed in viscosity among the investigated NaDES. In particular, the GCW-based formulations showed substantially higher viscosity values compared to the GLW-based systems. This behavior can be partially explained by their composition: water and lactic acid are liquid at ambient conditions, whereas glucose is a solid, and a higher content of solid components generally leads to increased resistance to flow. Among the three most viscous NaDES, GCW 2:2:4 exhibited the highest viscosity, consistent with its higher proportion of solid constituents. In addition, citric acid is known to significantly contribute to solution viscosity due to the presence of three carboxylic groups capable of forming multiple hydrogen bonds with surrounding molecules, unlike lactic acid or other less functionalized compounds.[18]

Figure 1 reports the FTIR-ATR spectra of the six NaDES investigated, with GCW-based systems shown on the left and GLW-based systems on the right.

All spectra exhibited a broad absorption band around the 3300–3400 cm^{-1} region, associated with O–H stretching vibrations and the extensive hydrogen-bond network involving the hydroxyl-rich NaDES components. An intense band was also observed in the carbonyl region, around 1700 cm^{-1}, and was attributed mainly to the C=O stretching vibrations of the carboxylic groups of citric and lactic acids. Additional signals in the 1200–1000 cm^{-1} region were assigned to C–O and C–OH stretching vibrations, including the band at approximately 1020 cm^{-1} associated with the pyranose ring of glucose.[19]

Comparison with the spectra of the individual components revealed changes in the position, width, and relative intensity of these bands. In the NaDES spectra, the O–H stretching envelope became broader and showed small formulation-dependent displacements relative to water, glucose, and the neat organic acids. Moreover, the relatively sharp C=O absorption of the neat organic acids near 1720 cm^{-1} broadened and displayed an apparent red shift toward approximately 1690–1710 cm^{-1} in the mixtures. Changes in band shape and relative intensity were also observed in the 1200–1000 cm^{-1} fingerprint region. Taken together, the broadening and displacement of the O–H and C=O bands and the changes in the fingerprint region indicate a modified local environment around the hydroxyl and carboxyl groups, consistent with the redistribution of intermolecular hydrogen bonding upon mixing of the components. Because some bands are broad and partially overlapping, these spectral changes are interpreted as evidence consistent with NaDES formation rather than as definitive proof based on a single diagnostic peak.

Following physicochemical characterization, the NaDES were evaluated for their ability to extract polyphenols (PC) from white and red grape pomace, and their performance was compared with that of a conventional solvent system (MeOH/HCl, 97:3). The extraction outcomes are summarized in Figure 2. Extraction performance was strongly substrate-dependent. For Wh-GP, NaDES formulations recovered approximately 3.0–6.8 mg GAE g^{-1}, less than MeOH/HCl (11.2 mg GAE g^{-1}). For Red-GP, however, NaDES recoveries were approximately 3.6–4.6 mg GAE g^{-1} and were therefore comparable to the MeOH/HCl value (4.6 mg GAE g^{-1}).

The selection of NaDES for subsequent experiments was based not only on extraction performance but also on their potential impact on sugar availability in the treated matrix, which could influence microbial activity during anaerobic digestion. Since the residual polyphenol content in the pomace was comparable regardless of the solvent used, two NaDES were selected as representative extremes in terms of glucose content: one with the highest glucose concentration and one with the lowest.

The amount of glucose present in 1 mL of each NaDES has been reported in Table 3.

Based on these values, GCW 2:2:4 (highest glucose content) and GLW 1:7:6 (lowest glucose content) were selected for the next experimental phase. These NaDES were used to treat both Wh-GP and Red-GP, which were subsequently employed as feedstocks for anaerobic fermentation tests at HRTs of 10 and 20 days.

3.2. Stoichiometric Estimation of NaDES-Derived COD and Sensitivity Analysis

The results of the stoichiometric model and the corresponding sensitivity analysis (Figure S2) show that NaDES carry-over can introduce a non-negligible COD load into the fermentation system, with GCW 2:2:4 contributing up to 0.93 $gCOD \cdot g^{-1}$ GP at V_{ret} = 1 $mL \cdot g^{-1}$, while GLW 1:7:6 contributed 0.34 $gCOD \cdot g^{-1}$ GP under the same conditions.

The sensitivity analysis showed a linear relationship between COD contribution and retained solvent volume. GCW exhibited a COD impact approximately 2.8 times higher than GLW, due to its higher glucose and citric acid content. Simulated solvent recovery demonstrated that even moderate recovery (50%) substantially reduces COD input, while high recovery (90%) nearly eliminates the effect. This suggests that solvent management strategies (washing, pressing, evaporation, or NaDES recycling) could effectively mitigate the metabolic shifts observed in the fermentation tests. The modeling results provide a plausible explanation for the drastic reduction in biogas production observed in NaDES-pretreated GP and support the hypothesis that the additional soluble carbon load alters microbial pathways, promoting acidogenesis and the accumulation of intermediates.

3.3. Biogas and Fatty Acid Production from Unpretreated Red-GP and Wh-GP

3.3.1. Biogas Production. A marked difference was observed between Wh-GP and Red-GP in terms of biohydrogen, biomethane, and total biogas production, with methane and hydrogen yields reported in Figure 3.

Wh-GP exhibited higher overall biogas yields, reaching 183.0 and 250.0 L $kgVS^{-1}$ at HRTs of 10 and 20 days, respectively; whereas Red-GP produced lower total biogas volumes,

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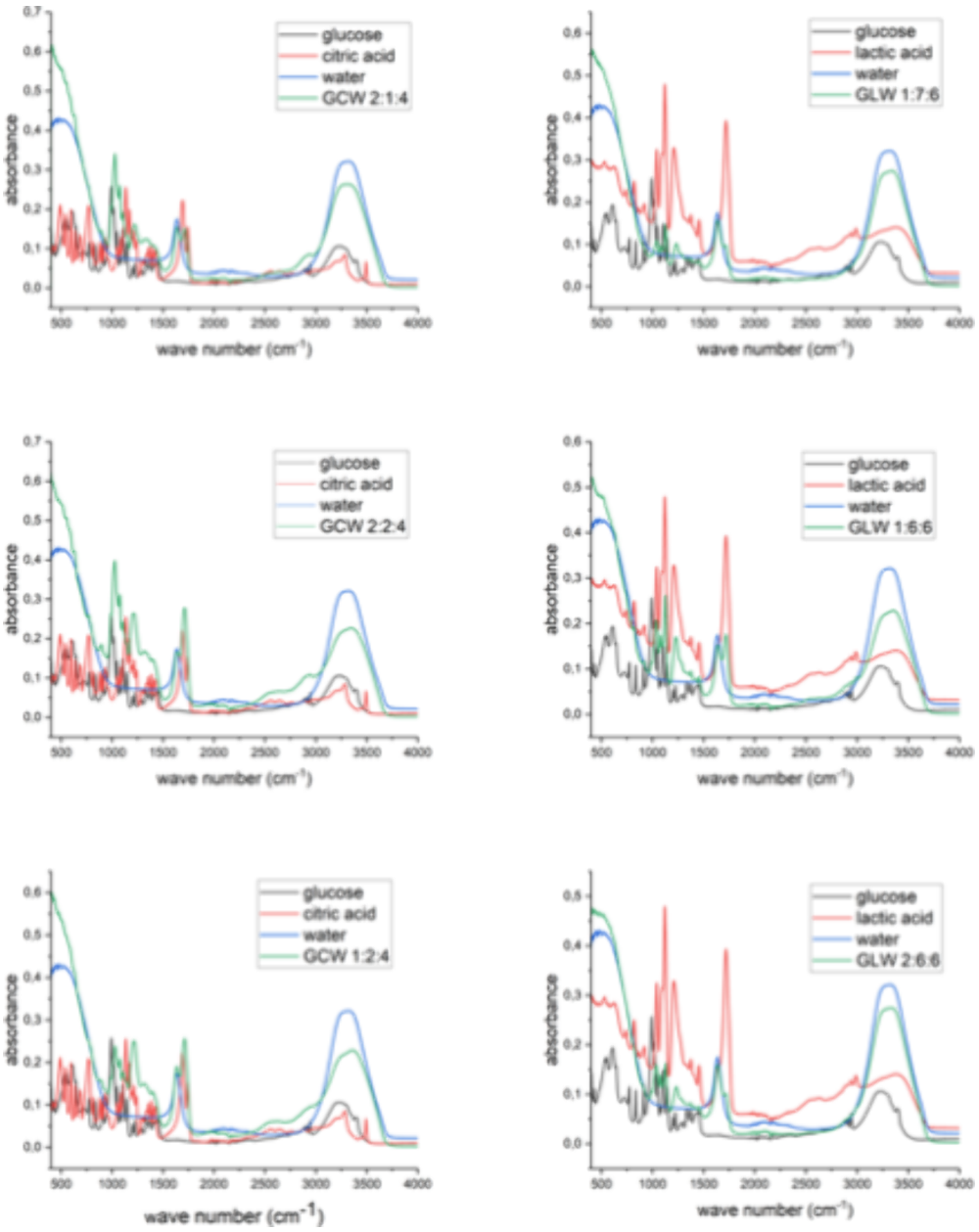

Figure 1. FTIR-ATR graphs of the studied NaDES.

amounting to approximately 85.0 and 220.0 L $kgVS^{-1}$ at the same HRTs. Despite the lower total gas production, Red-GP showed substantially higher specific methane and hydrogen yields. At an HRT of 10 days, hydrogen production from Red-GP reached approximately 33.0 L $kgVS^{-1}$, while methane was detected only in trace amounts (≈2.0 L $kgVS^{-1}$). At an HRT of 20 days, Red-GP achieved methane and hydrogen production of 176.0 and 0.3 L $kgVS^{-1}$, respectively (Figure 3). In contrast, Wh-GP exhibited limited methane production, with values of 2.5 L $kgVS^{-1}$ at an HRT of 10 days and 89.0 L $kgVS^{-1}$ at an HRT of 20 days (Figure 3). Overall, these results indicate that Red-GP resulted in the production of biogas with higher energy content despite its lower total volume.

This apparent discrepancy highlights the importance of distinguishing between total gas production and carbon conversion pathways during anaerobic fermentation. The higher biogas volumes associated with Wh-GP may be related to differences in substrate composition arising from the winemaking process. Wh-GP is separated from the must prior to alcoholic fermentation and therefore retains a larger fraction of readily fermentable substrates, including soluble carbohydrates. These compounds are rapidly metabolized by acidogenic microorganisms, promoting intense fermentative activity and resulting in the production of large amounts of carbon dioxide and intermediate metabolites such as VFAs and lactic acid. Under such conditions, the process tends to be dominated by acidogenesis, which favors gas generation but does not necessarily translate into efficient conversion toward reduced gaseous products such as methane and hydrogen. This trend was evident at both HRTs of 10 and 20 days. Conversely, Red-GP undergoes prolonged

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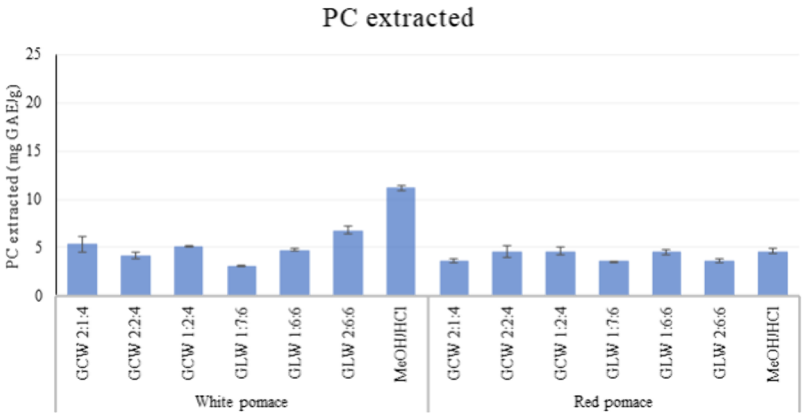

Figure 2. Total phenolic content extracted from white and red grape pomace using different NaDES formulations and conventional MeOH/HCl.

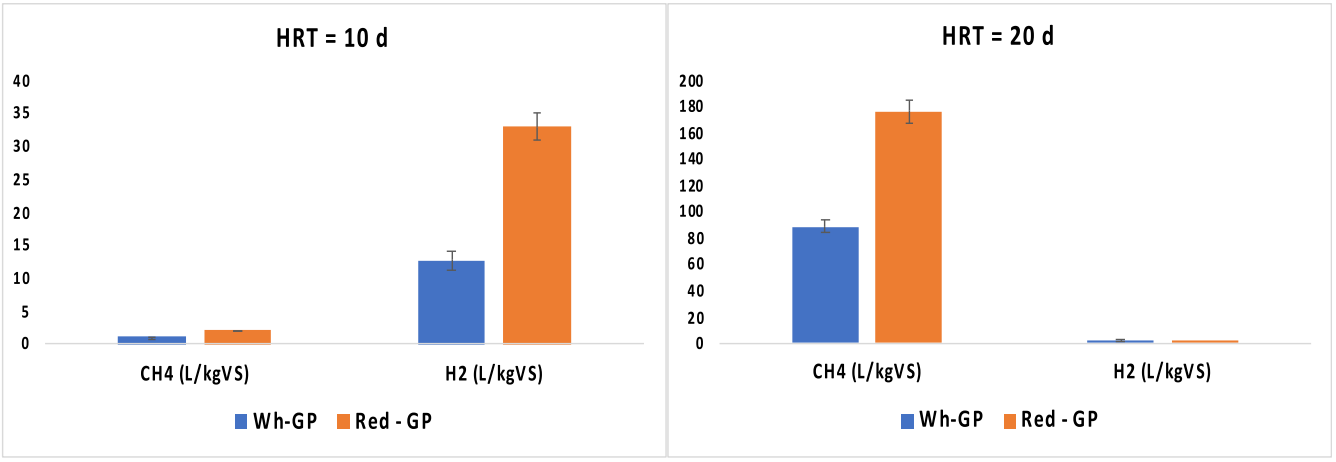

Figure 3. Methane and hydrogen production for Red-GP and Wh-GP at HRTs of 10 and 20 days. Data are reported as mean ± standard deviation during the final two HRTs.

contact with fermenting must during vinification, leading to a depletion of easily degradable sugars and to an enrichment in more recalcitrant components, such as lignocellulosic structures and phenolic compounds.[20,21] As a result, anaerobic fermentation of Red-GP proceeds at a slower rate and yields lower total gas volumes. However, the gas produced under these conditions exhibits a higher proportion of methane and hydrogen, indicating a more effective routing of carbon toward reduced end-products. This suggests that, despite lower overall biodegradability, Red-GP favors metabolic pathways associated with acetogenesis and methanogenesis rather than extensive acidogenic activity. However, mono- and polysaccharides were not specifically quantified in the present study; therefore, this interpretation should be regarded as a plausible explanation supported by previous literature rather than as a direct mechanistic demonstration.

Moreover, the role of polyphenolic compounds appears to be particularly relevant in this context. Although polyphenols are often cited as inhibitors of AD, their effect is strongly concentration-dependent and matrix-specific. For example, in a previous research work on AD of by-products from olive oil production, it was demonstrated that the high polyphenol content in olive mill wastewater of around 55–60 mg GAE g^{-1} led to a strong reduction of methanogenic activity.[22] Consequently, specific pretreatments were necessary to reduce their concentrations in the reaction medium. On the contrary, at the concentrations typically encountered in GP, polyphenols (4–12 mg g^{-1}, Table 1) may not completely suppress microbial activity but rather exert a selective pressure on the microbial consortium. A previous work by Battista et al.[9] on Red-GP confirmed that methane performance was better without polyphenol extraction, demonstrating that their inhibition effect starts when their concentration is over 2–5 g L^{-1} in the reaction medium.[23,24] Considering the specific content of polyphenolic compounds in Red-GM (Table 1) and the specific operative conditions of the different tests, this value was reached neither for the semi-continuous tests in this work.

The behavior observed in this study is consistent with previous findings reported for GP and other agro-industrial residues. Several authors have reported that substrates rich in readily fermentable carbohydrates tend to promote acidogenic pathways, resulting in high gas production dominated by carbon dioxide and VFAs, but relatively low methane yields. In contrast, more recalcitrant substrates often exhibit lower overall gas

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production, but higher methane fractions due to a more balanced progression toward acetogenesis and methanogenesis. Martinez et al.[5] reported similar trends during the AD of GP within an integrated biorefinery scheme, where the removal of polyphenols did not lead to a proportional increase in methane production and process performance was largely governed by substrate composition and operational parameters. Likewise, Neri et al.[25] highlighted that high concentrations of easily degradable organic matter can induce excessive acidification, limiting methane formation despite high biogas generation. The selective rather than purely inhibitory role of polyphenols has also been discussed in the literature. Castellanos-Gallo et al. and Karastergiou et al.[1,26] noted that phenolic compounds may influence microbial community structure and fermentation pathways without necessarily causing complete process inhibition, particularly when present at moderate concentrations.

3.3.2. Short and Medium Fatty Acid Production. The production and distribution of VFAs and caproic acid from untreated Wh-GP and Red-GP were markedly affected by both substrate type and operational parameters, HRT, and OLR (Figure 4). At an HRT of 10 days, Wh-GP achieved a total VFA yield of 38% of the inlet COD, more than double that obtained from Red-GP (15%). Extending the HRT to 20 days led to a substantial increase in VFA accumulation for both substrates, with Wh-GP reaching 81.5% and Red-GP 31.5%.

Across both HRTs, Wh-GP consistently exhibited higher total VFAs and caproic production than Red-GP (Figure 4). This trend can be primarily attributed to differences in substrate composition arising from the vinification process. As previously discussed, Wh-GP is separated from the must prior to alcoholic fermentation and therefore retains a higher fraction of readily fermentable substrates, including soluble carbohydrates and easily hydrolyzable organic matter.[20] These compounds are rapidly metabolized by acidogenic microorganisms, promoting intense acidogenesis and leading to elevated fatty acid accumulation. In contrast, Red-GP undergoes prolonged contact with fermenting must, resulting in the depletion of simple sugars and an enrichment in more recalcitrant lignocellulosic fractions. This interpretation is consistent with previous studies on GP valorization. Martinez et al.[5] reported that acidogenic fermentation of Wh-GP resulted in higher VFA yields compared to red-GP, mainly due to differences in residual carbohydrate content. Similarly, Neri et al.[25] reported that substrates rich in easily fermentable carbon favor VFA accumulation.

Focusing on caproic acid, the medium fatty acid with the highest economic value, its formation was detected for both substrates and increased with longer HRTs, indicating the occurrence of chain-elongation reactions under the tested conditions. For Wh-GP, caproic acid increased from 4.5% at an HRT of 10 days to 14.5% at an HRT of 20 days, becoming a significant fraction of the total VFAs. In contrast, Red-GP showed substantially lower caproic acid yields, reaching only 2% at the longer HRT. The enhanced caproic acid production observed for Wh-GP can be attributed to the greater availability of short-chain fatty acids, such as acetic and butyric acids, which act as key precursors for chain elongation via reverse β-oxidation pathways. These observations are in agreement with the work by Battista and Bolzonella, (2024), who demonstrated that specific concentrations of short-chain carboxylates and sufficiently long HRTs are essential to promote medium-chain fatty acid production. Similar conclusions reported by Wang et al.[28] showed that chain elongation from lignocellulosic residues is strongly dependent on carbon availability and process stability. Conversely, the limited caproic acid formation observed for Red-GP suggests that the lower availability of fermentable intermediates and the more recalcitrant nature of the substrate constrained the extent of chain-elongation reactions.

Finally, it is also important to highlight the effect of the HRT and OLR on VFAs. Increasing the HRT from 10 to 20 days and reducing the OLR resulted in a pronounced enhancement of VFA production for both substrates, highlighting the critical role of these parameters in acidogenic fermentation of GP. Longer HRTs provide microorganisms with sufficient time to complete hydrolysis and acidogenesis, which is particularly important when processing complex and partially lignified substrates. This effect was especially evident for Wh-GP, where total VFA yields more than doubled when extending the HRT. In addition, extended HRT reduces the risk of washout of slow-growing acidogenic and chain-elongating microorganisms, thereby promoting the development of more stable and specialized microbial consortia. Meegoda et al.[29] identified hydrolysis as the rate-limiting step in AD of complex substrates, emphasizing that short HRT can severely constrain overall process performance. Arslan et al.[30] further demonstrated that longer retention times favor secondary metabolic compounds, including medium fatty acids from chain elongation. Comparable trends were also reported by Tang et al.,[31] who observed significantly higher VFA accumulation at longer HRTs during the acidogenic fermentation of food and

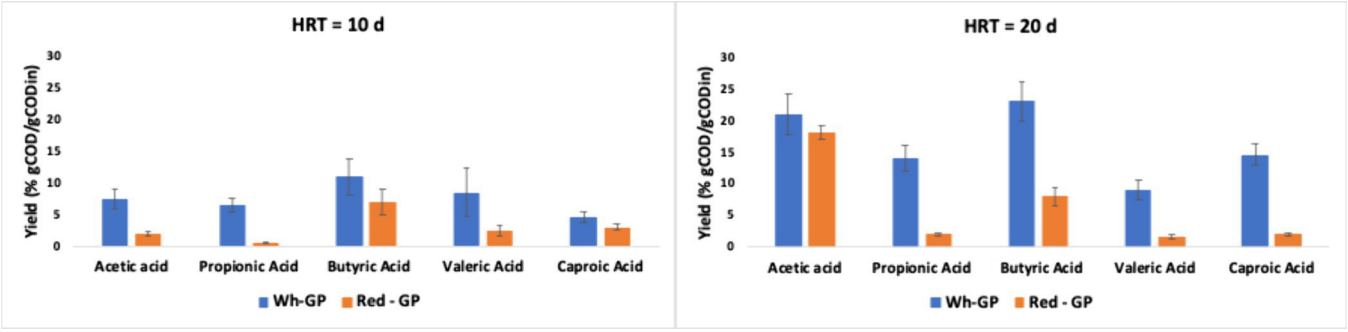

Figure 4. Short and medium fatty acid production from not pretreated Wh-GP and Red-GP at HRTs of 10 and 20 days. Data are reported as mean ± standard deviation during the final two HRTs.

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agro-industrial wastes. Overall, these results confirm that HRT is a key operational parameter governing both the quantity and composition of VFAs, often exerting a stronger influence than substrate type alone.

3.4. Biogas and Fatty Acid Production from Extracted Red-GP and Wh-GP

3.4.1. Estimated Contribution of NaDES Carry-Over to Influent COD Composition. The stoichiometric model developed in this study estimated the potential contribution of NaDES carry-over to the composition of the influent COD. The model integrates the concentrations of glucose and organic acids in each NaDES formulation with their theoretical COD factors, allowing estimation of the COD introduced per gram of pretreated GP (Supplementary Material S1).

For GCW 2:2:4, the combination of a high glucose concentration (0.50 g mL^{-1}) and the stoichiometric presence of citric acid results in a COD contribution of approximately 0.93 gCOD g^{-1} GP at a retained solvent volume of 1 mL g^{-1}. In contrast, GLW 1:7:6, characterized by lower glucose content (0.07 g mL^{-1}) but substantial lactic acid, introduces approximately 0.34 gCOD g^{-1} GP under the same conditions. These values demonstrate that NaDES carry-over represents a non-negligible and readily biodegradable carbon input, capable of influencing microbial metabolism independently of the structural effects of pretreatment. The sensitivity analysis (Supplementary Material S2) further shows that COD contribution increases linearly with the retained solvent volume and decreases proportionally with solvent recovery efficiency. Even moderate recovery (50%) substantially reduces the COD load, while high recovery (90%) nearly eliminates it. This behavior confirms that the observed shifts in fermentation performance are not solely attributable to polyphenol removal or substrate modification but also to the direct metabolic assimilation of NaDES components such as glucose, lactate, and citrate.

This interpretation is consistent with the experimental results: reactors fed with NaDES-pretreated GP exhibited accumulation of acidic soluble intermediates and suppression of methanogenesis. Similar responses have been reported in systems supplemented with lactate or other easily fermentable substrates, where excess electron donors promote acidogenesis and chain-elongation processes.[32] The high lactate concentrations measured in GLW-fed reactors (up to ∼10–11 gCOD L^{-1} at an HRT of 10 days, Table 4) further support the occurrence of solvent carry-over and confirm that NaDES components actively participate in the metabolic network.

Overall, these findings indicate that NaDES cannot be considered biologically inert extraction media in integrated biorefinery schemes. Rather, NaDES carry-over introduces a solvent-derived fraction within the influent COD, thereby altering its composition, biodegradability, and subsequent product distribution. Effective integration of NaDES extraction with anaerobic fermentation therefore requires careful solvent management and downstream process adaptation. Nevertheless, the present experimental design did not allow the individual effects of polyphenol removal, NaDES carry-over, and extraction-induced modifications of the GP matrix to be fully disentangled. Simple removal of residual NaDES by washing would also remove endogenous soluble organic matter from the GP, thereby altering both the total COD and its composition. Consequently, dedicated factorial controls would be required to independently quantify the effects of polyphenol removal, NaDES carry-over, and extraction-induced matrix modifications. The soluble organic fraction was characterized in terms of fatty acids and lactate, whereas total soluble COD, residual glucose, and citrate were not systematically quantified. Therefore, the relative contributions of GP-derived soluble organic matter and NaDES carry-over could not be conclusively distinguished.

3.4.2. Biogas Production. As anticipated, polyphenol extraction with NaDES resulted in a marked suppression of biogas production for both Wh-GP and Red-GP. When expressed as cumulative production, total biogas yields from extracted substrates ranged between 3.4 and 16.9 L $kgVS^{-1}$, regardless of pomace type, NaDES formulation, or retention time. These values are approximately one order of magnitude lower than those obtained from untreated GP under comparable conditions, where total biogas yields ranged from approximately 85 to 250 L $kgVS^{-1}$, as previously reported. In addition to the substantial quantitative reduction, NaDES pretreatment also caused a severe deterioration in biogas quality. Gas composition was overwhelmingly dominated by CO_2, accounting for 96–100% of the total biogas in nearly all tested conditions. Methane was detected only sporadically and at very low absolute yields (≤0.4 L $kgVS^{-1}$), while hydrogen was almost always negligible. Overall, these results clearly indicate that polyphenol removal alone would not necessarily be expected to promote methanogenesis or hydrogenogenesis under the tested conditions.

Due to the intrinsic viscosity and strong solvent–matrix interactions, a non-negligible amount of NaDES remained associated with the pretreated GP after extraction, as previously reported, resulting in the continuous introduction of NaDES-derived compounds during reactor feeding. Although NaDES are often described as "green" and biodegradable, their components, such as sugars and organic acids, can strongly influence the processes when supplied at elevated local concentrations.[13,33] This interpretation is consistent with the simultaneous observation of enhanced acidogenic activity under specific conditions, discussed in the following VFA section. From a broader perspective, these findings align with emerging literature indicating that deep eutectic solvents and NaDES are not biologically inert. Depending on their composition and residual concentration, they may exert selective or inhibitory effects on anaerobic microbial consortia, particularly on strictly anaerobic and slow-growing microorganisms such as methanogens.[13] Another study has reported that, unless adequately removed or diluted, residual NaDES can compromise downstream biological conversion processes despite improving upstream extraction efficiency. Specifically, metagenomic analysis demonstrated that the hydrolytic-acidogenic bacteria were

Table 4. Lactic Acid Concentrations in the Reaction Medium Tests Performed with Extracted GP

	NaDES	HRT (d)	lactic acid concentration (gCOD L^{-1})
Extr-Wh-GP	GCW 2:2:4	10	0.35 ± 0.07
		20	1.85 ± 0.25
	GLW 1:7:6	10	11.36 ± 0.93
		20	6.22 ± 1.56
Extr-Red-GP	GCW 2:2:4	10	0.28 ± 0.20
		20	1.03 ± 0.05
	GLW 1:7:6	10	11.18 ± 0.44
		20	5.00 ± 1.69

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enriched after NADES treatments, favoring VFA production and compromising biogas yield.[34]

This highlights the importance of carefully balancing upstream extraction benefits with downstream biological compatibility when designing integrated biorefinery schemes.

3.4.3. Short and Medium Fatty Acid Production. The production and distribution of VFAs and MCFAs from extracted GP were strongly influenced by both the choice of the employed NaDES and the HRT, showing markedly different trends compared to untreated substrates. Figures 5 and 6 showed the VFA and caproic acid yields for Extr-Wh-GP and Extr-Red-GP with the two NADES combinations which had the best polyphenol extraction.

Overall, polyphenol extraction substantially modified acidogenic performance, leading to lower and more variable fatty acid yields, with a clear dependence on NaDES composition and operating conditions. Across all tested conditions, higher VFA concentrations and yields were systematically achieved at HRT = 20 days, whereas HRT = 10 days resulted in limited acid accumulation for both Extr-Wh-GP and Extr-Red-GP (Figures 5 and 6). This confirms that, following NaDES pretreatment, longer HRT and lower OLR are essential to stabilize acidogenic fermentation and allow effective conversion of the residual organic matter, when the substrates contain residual solvents from previous upstream processes. Moreover, a pronounced effect of NaDES composition on VFA speciation was observed. Both Wh-GP and Red-GP pretreated with GCW 2:2:4 showed lower VFA yields than GLW 1:7:6 for both the HRT 10 and 20 days. Specifically, the total fatty acid yields for Extr-Wh-GP were 0.71 and 6.49% w/w at HRTs of 10 and 20 d, respectively, with GCW 2:2:4, and of 2.10 and 40.07% w/w at HRTs of 10 and 20 d, respectively, with GLW 1:6:7. While the total fatty acid yields for Extr-Red-GP were 0.39 and 16.74% w/w at HRTs of 10 and 20 d, respectively, with GCW 2:2:4, and of 3.56 and 46.39% w/w at HRTs of 10 and 20 d, respectively, with GLW 1:6:7.

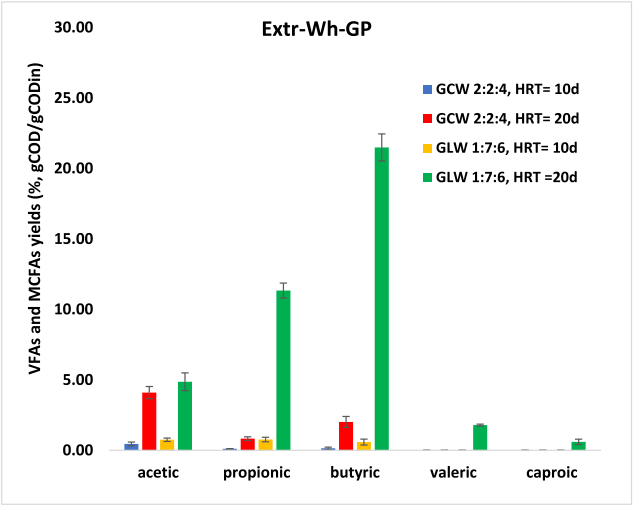

Figure 5. VFA and caproic acid yields for Extr-Wh-GP. Data are reported as mean ± standard deviation during the final two HRTs.

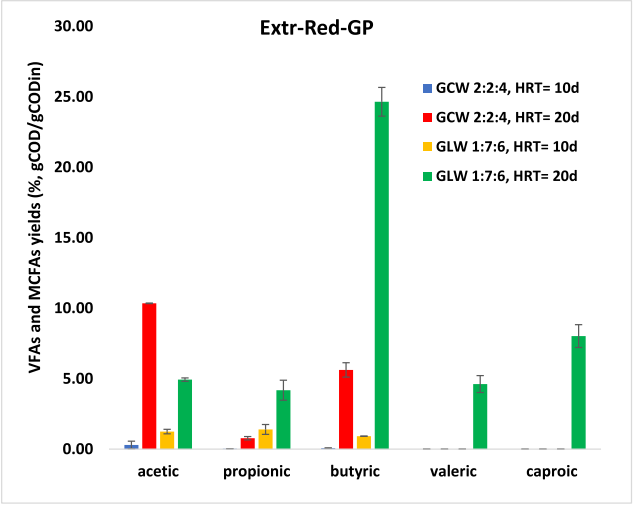

Figure 6. VFA and caproic acid yields for Extr-Red-GP. Data are reported as mean ± standard deviation during the final two HRTs.

Regarding the fatty acid profile, acetic acid was the dominant one, accounting for more than 50% of total VFAs and MCFAs when GCW 2:2:4 was used for pretreatment. This behavior is coherent with the biochemical role of citric acid, which is readily metabolized through central metabolic pathways and mainly supports acetate formation.[35] Conversely, pretreatments with GLW 1:7:6 promoted a shift toward more reduced VFAs, including propionic and butyric acids, and formation of valeric and caproic acids exclusively at HRT = 20 days. This shift can be attributed to the presence of lactic acid in the GLW formulation, which is known to act as an effective electron donor and precursor for reductive pathways, favoring chain elongation through reverse β-oxidation mechanisms, as discussed in previous works on carboxylate-platform biorefineries.[5,27]

The lactate concentrations measured in the fermentation medium (Table 4) further support this interpretation, even if HRT emerged as a fundamental parameter to allow the lactic acid entrance in the chain-elongation loop. Reactors fed with GLW-pretreated pomace exhibited high lactate levels at HRT = 10 days of around 10 gCOD L^{-1}, which coincided with poor VFA accumulation and strongly suppressed both biogas production, as previously discussed, and the chain-elongation process. In fact, under these conditions, the excessive availability of lactic acid relative to the active biomass may have induced metabolic imbalances and acid stress, ultimately limiting its conversion into VFAs and MCFAs, as also reported for lactate-mediated chain-elongation systems operating at high specific microbial loading rates.[36] While, at HRT = 20 days, lactate concentrations decreased to approximately 5–6 gCOD L^{-1}, a range more compatible with a first gradual microbial conversion into propionate and butyrate, and then into caproic acid. Specifically, it was detected for Extr-Red-GP, reaching a yield of about 8% w/w (Figure 4). These results indicate that NaDES pretreatment can modulate VFA distribution and enable chain elongation under specific conditions but does not inherently enhance caproate yields compared to untreated GP. Indeed, as discussed in Section 3.1.2, the highest overall caproate production in this study was achieved using untreated Wh-GP at HRT = 20 days. This suggests that substrate chemical composition, metabolic balance, and operating conditions exert a stronger influence on chain-elongation performance than polyphenols removal alone, in agreement with previous studies on GP and other lignocellulosic residues.[5,25]

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Table 5. COD Distribution for the 12 Semi-continuous Fermentation Runs

substrate	NaDES	HRT (d)	COD to CH_4 (%)	COD to H_2 (%)	COD to VFAs and MCFAs (%)	residual and unaccounted COD (%)
Wh-GP	–	10	0.2	0.6	38.0	61.2
Wh-GP	–	20	17.9	0.1	81.5	0.5
Red-GP	–	10	0.4	1.7	15.0	82.9
Red-GP	–	20	35.4	0.0	31.5	33.1
Extr-Wh-GP	GCW 2:2:4	10	≈0.0	≈0.0	0.7	99.3
Extr-Wh-GP	GCW 2:2:4	20	≈0.0	≈0.0	6.5	93.5
Extr-Wh-GP	GLW 1:7:6	10	≈0.0	≈0.0	2.1	97.9
Extr-Wh-GP	GLW 1:7:6	20	≈0.0	≈0.0	40.1	59.9
Extr-Red-GP	GCW 2:2:4	10	≈0.0	≈0.0	0.4	99.6
Extr-Red-GP	GCW 2:2:4	20	≈0.0	≈0.0	16.7	83.3
Extr-Red-GP	GLW 1:7:6	10	≈0.0	≈0.0	3.6	96.4
Extr-Red-GP	GLW 1:7:6	20	≈0.0	≈0.0	46.4	53.6

Analyzing better the influence of HRT, the comparison between HRT = 10 and 20 days highlights the dominant role of this parameter on VFA and caproic acid yields from Extr-Wh-GP and Extr-Red-GP. Longer HRTs provide sufficient time for hydrolysis and acidogenesis of the GP, partially composed by recalcitrant matrix remaining after NaDES extraction, while reducing the risk of washout of slow-growing acidogenic and chain-elongating microorganisms. Moreover, as emphasized by Table 4, longer HRTs provided the time to lactic acid to enter in the chain-elongation loop, reducing its accumulation in the reaction medium. Similar trends have been reported for the acidogenic fermentation of complex agro-industrial residues, where extended retention times favor higher VFA accumulation and the establishment of secondary metabolic pathways.[29,30,37]

Finally, it is important to emphasize the difference between the two types of extracted GP. The results showed a better performance of Extr-Red-GP, which can be interpreted as a continuation of the qualitative behavior already observed and discussed for untreated substrates.[38] After NaDES extraction, this intrinsic trend became more evident, indicating that the extraction step did not disrupt, but rather emphasized, the inherent structural and compositional features of Red-GP.[39] Under conditions combining lactate availability and extended HRT, Extr-Red-GP sustained a more gradual and balanced acidogenic process, favoring the progressive utilization of intermediates and creating conditions that promoted the chain-elongation pathways, including caproate formation. Conversely, Extr-Wh-GP promoted faster acidogenic reactions which, despite yielding higher total VFA concentrations in some cases, were less effective in steering the system toward medium-chain fatty acid production. Overall, these results indicate that the structural and compositional features of Red-GP inherently favor metabolic stability.

3.5. Estimated COD Distribution among Fermentation Products

To provide a common basis for comparing the 12 fermentation runs, the distribution of the influent COD among methane, hydrogen, and the measured fatty acids was estimated. Methane and hydrogen yields, originally expressed on a VS basis, were converted into COD equivalents, whereas fatty acid yields were already expressed relative to the influent COD. Carbon dioxide was not included because it does not contribute to COD. The fraction not recovered as methane, hydrogen, or measured fatty acids was reported as residual and unaccounted COD, which may include unconverted soluble and particulate organic matter, microbial biomass, lactate, and other non-quantified fermentation products. The estimated COD distribution is summarized in Table 5.

4. CONCLUSIONS

This study demonstrated that the anaerobic fermentation of GP is strongly governed by intrinsic substrate composition, operational parameters, and the eventual presence of pretreatments. No pretreated Wh-GP exhibited a more reactive acidogenic behavior with higher VFA and gas production, whereas Red-GP showed a more selective metabolic pattern, producing methane and hydrogen-enriched biogas. These differences confirm that substrate origin fundamentally shapes fermentation pathways.

NaDES-based polyphenol extraction did not simply enhance or inhibit AD; rather, it altered the distribution of carbon products during fermentation. NaDES successfully extracted polyphenols from both GP, although their performance depended on the solvent formulation and substrate type. Extraction yields were comparable to those obtained with conventional MeOH/HCl for Red-GP, whereas lower recoveries were observed for Wh-GP. In contrast, the solvent-derived GP within the fixed influent COD altered its composition and was associated with strongly reduced methane and hydrogen production and a composition- and HRT-dependent accumulation of soluble intermediates. The proposed stoichiometric framework highlights the potential relevance of solvent-derived organic inputs. These findings emphasize the importance of considering process compatibility and solvent management when integrating extraction and fermentation within grape pomace biorefineries.

Future studies should address process scale-up and include a dedicated techno-economic assessment accounting for the costs of NaDES ingredients and preparation, solvent recovery and recycling, energy requirements, downstream product purification, and the economic value of the recovered polyphenols and fermentation products.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.6c06566.

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Stoichiometric calculations for NaDES-derived COD and sensitivity analysis of retained solvent volume and recovery efficiency (DOCX)

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Notes

The authors declare no competing financial interest.

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