

Article

Impact of Chemical and Physical Pretreatment on Methane Potential of Peanut Shells

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Abstract: The request for alternative sources of energy has led to evaluating untapped routes for energy production, such as using abundant and low-cost waste materials, e.g., lignocellulosic wastes, as the substrate for biological processes aimed at biofuel production. This study focused on peanut shells (PS) valorisation via anaerobic digestion (AD). Two emerging pretreatments, i.e., organosolv and ultrasounds, were investigated to unlock the full AD potential of PS. The impact of a substrate-to-solvent ratio in organosolv pretreatment was investigated (i.e., 1:5 vs. 1:10 vs. 1:20). Different exposure times were tested for ultrasound pretreatment, corresponding to applied energy densities of 30,000, 12,000, and 6000 kJ/kg VS, respectively. Organosolv pretreatment achieved the maximal polyphenol solubilisation, i.e., 4.90 mg/g TS, when increasing the substrate-to-solvent ratio, whereas methane production did not benefit from the pretreatment, being comparable with that of raw PS at most (i.e., 55.0 mL CH₄/g VS). On the other hand, ultrasounds mainly affected sugar solubilisation (up to 37.90 mg/g TS), enhancing methane production up to an extra 64%, achieved with the highest energy density. The organosolv route would benefit from further downstream steps to recover the biomolecules released in the liquid fraction, whereas ultrasounds pretreatment provided a slurry suitable for direct AD.

Keywords: lignocellulosic waste; ultrasound pretreatment; organosolv pretreatment; anaerobic digestion; polyphenols; sugars



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1. Introduction

The depletion of fossil fuels has encouraged the development of alternative strategies to increase the energy supply from green resources [1]. Residual biomasses are one of the most produced wastes from industrial processing, farming, and forestry. Such wastes represent an issue for natural and urban environments due to their abundance and footprint [2,3]. Despite most biomasses still being employed for house heating or direct incineration [4,5], biological processes such as anaerobic digestion (AD) have been largely exploited in the last decades to valorise such wastes [6,7].

The AD process has developed from an initial concept of a strategy to mitigate the impact of mainly wastewater streams and other pollutants to a real bioenergy factory utilising the most varied substrates for energy production and high-value product recovery [8–10]. AD digestion is carried out by different groups of microorganisms that act synergically to degrade complex organic substrates in four sequential stages, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis, to produce biogas, i.e., a gas mixture of carbon dioxide and methane, as the final product [11]. In particular, lignocellulosic wastes (LWs) are abundant biomass generated throughout the world. LWs are an ideal substrate for energy production through AD due to their high carbon content, carbohydrate richness, and low/null cost of supply [12]. Nonetheless, the high lignin content and the presence of other non-structural components can limit the biomethane potential of LWs [13]. The

structural components of LWs are cellulose, hemicellulose, and lignin, with the latter being one of the limiting factors for the efficient anaerobic degradation of LWs [14]. Depending on the specific LWs, different pretreatment strategies have been investigated to maximise the efficiency of AD and achieve high biogas production, high methane content in biogas, and limited digestate production [15]. Pretreatments are meant to enhance the enzymatic activity of hydrolytic bacteria, which is generally the limiting factor in fermentative processes involving solid substrates such as LWs [16].

Pretreatments are usually divided into physical, chemical, physicochemical, and biological methods. Despite biological pretreatments such as fungi or enzymes recently being explored, these strategies usually require long exposure times and high costs, resulting in poor feasibility at a large scale [17]. Therefore, this study focused on physical, i.e., ultrasound, and chemical, i.e., organosolv, pretreatments. Ultrasounds have the advantage of being a chemical-free, easy-handling pretreatment, capable of interacting with all the structural components of the biomass, resulting in high hydrolysis rates [14,18]. Ultrasounds have been largely employed to enhance the biodegradation of liquid substrates, e.g., digestate, slurry, and sludge, but only a few studies tested this pretreatment on hard–solid substrates, such as LWs [19,20]. On the other hand, organosolv pretreatment has been reported as one of the most selective pretreatments, due to its impact on the lignin content. Organosolv pretreatment leads to the structural deconstruction of LWs through different steps, which involve the rupture of internal lignin bonds and lignin-hemicellulose bonds, hydrolysis of glycosidic bonds between cellulose and hemicellulose, and degradation of pentose and hexose sugars [21]. In addition, organic solvents such as ethanol and methanol can be easily recovered and reused, thus contributing to the favouring of organosolv over other chemical pretreatments [22].

To the best of the authors' knowledge, this is the first study to investigate the impact of ultrasound and organosolv pretreatment on peanut shells (PS). In this study, different ultrasound pretreatment exposure times were investigated, i.e., 1, 2, and 5 min, resulting in 6000, 12,000, and 30,000 kJ/kg VS, respectively, as the applied energy density (E_d). Organosolv pretreatment was performed using methanol (MeOH) as the organic solvent, with the objective to assess the most effective substrate-to-solvent ratio, i.e., 1:5, 1:10, and 1:20, to disrupt PS. The effectiveness of the two pretreatments was evaluated in terms of sugar and polyphenol release and methane production. Raw, ultrasound, and organosolv-pretreated PS were subjected to mesophilic AD to evaluate their methane potential. The experimental results showed that ultrasound pretreatment can enhance methane production from PS up to 64%, achieving 78.6 mL CH_4 /g VS. On the other hand, organosolv pretreatment did not lead to an increment in methane production but enabled the solubilisation of high-value biomolecules, i.e., carbohydrates and polyphenols, that can be further valorised in a circular bioeconomy perspective.

2. Materials and Methods

2.1. Substrate Preparation and Inoculum

The PS were obtained from a grocery store located in the Lazio region (Italy), which purchased shelled peanuts of Egyptian origin. The edible part was separated from the shell and the skin. The shells were crushed and sieved to select a particle size between 1.0 and 2.5 mm. The PS were stored in plastic bags at 4 °C before being pretreated and subjected to AD. A digestate from buffalo manure (DBM) collected from a full-scale anaerobic digester located in Capaccio (Campania region, Italy) was used as the inoculum for the biochemical methane potential (BMP) tests [23].

2.2. Organosolv Pretreatment

The organosolv pretreatment was performed in a stainless steel reactor (Sigma-Aldrich, Darmstadt, Germany) using a 50% (*v/v*) water–methanol solution. Sulfuric acid (0.01 M) was used as the catalyst. Different substrate-to-solvent ratios were tested, i.e., 1:20, 1:10, and 1:5, to evaluate the amount of solvent required to break down the PS. The raw PS

were carefully mixed with the organic solvent in a glass beaker and then transferred into the stainless steel reactor. The organosolv pretreatment was performed at 130 °C with an exposure time of 60 min. Table 1 summarises the organosolv pretreatment conditions investigated in this study. After pretreatment, the liquid fraction was separated from the solid residues and stored at −20 °C for carbohydrate and polyphenol analysis. The pretreated PS were rinsed with abundant water to remove any residue of the organic solvent and dried at 45 °C before undergoing AD.

Table 1. Experimental conditions tested for the organosolv pretreatment of peanut shells.

Pretreatment Condition	Solvent	Catalyst	Substrate/Solvent (g/g)	Temperature (°C)	Exposure Time (min)
1	50% Methanol	0.01 M H ₂ SO ₄	1:20	130	60
2	50% Methanol	0.01 M H ₂ SO ₄	1:10	130	60
3	50% Methanol	0.01 M H ₂ SO ₄	1:5	130	60

2.3. Ultrasounds Pretreatment

The ultrasounds pretreatment was performed by applying different E_d to PS. Distilled water was used as the medium for ultrasonic wave diffusion, keeping a substrate-to-medium ratio of 1:20. A preliminary test was conducted to compare the effect of different devices, i.e., an RK52 ultrasonic bath (Bandelin, Berlin, Germany) and an HD4200 ultrasonic device (Bandelin, Berlin, Germany) equipped with a TS113 titanium flat probe (diameter = 13 mm; length = 130 mm) (Bandelin, Berlin, Germany). The selected ultrasonic bath works at a frequency (f) of 35 kHz, whereas f was 20 kHz for the ultrasonic probe. The exposure time was calculated to guarantee the same E_d for the two devices. Three different E_d were compared, i.e., 5800, 11,600, 23,200 kJ/kg VS. The liquid fraction after pretreatment was analysed to evaluate carbohydrate and polyphenol solubilisation.

Based on carbohydrate and polyphenol solubilisation obtained from the preliminary screening, the highest-performing device was selected. The ultrasounds pretreatment was repeated by treating 2 g VS of PS in 40 mL of distilled water. The nominal power (P) and amplitude were 200 W and 100%, respectively. The exposure time, i.e., 1, 2, and 5 min, was calculated as previously described by Zou et al. [24] to guarantee an E_d of approx. 6000 (6 k), 12,000 (12 k), and 30,000 (30 k) kJ/kg VS, respectively. The experimental conditions performed for ultrasounds pretreatment are reported in Table 2. The liquid fraction from ultrasounds pretreatment was sampled for carbohydrate and polyphenol analysis. In the case of ultrasounds pretreatment, the slurry (i.e., solid and liquid fraction) was subjected to AD.

Table 2. Experimental conditions tested for the ultrasounds pretreatment of peanut shells.

Pretreatment Condition	Medium	Substrate/Medium (g/g)	Nominal Power (W)	Exposure Time (min)	Energy Density (kJ/kg VS)
1	H ₂ O	1:20	200	1	6000
2	H ₂ O	1:20	200	2	12,000
3	H ₂ O	1:20	200	5	30,000

2.4. Biochemical Methane Potential Tests and Model Fitting

The BMP tests were performed using 250 mL serum bottles as bioreactors. An amount of 2 g VS from raw or organosolv-pretreated PS was mixed with the inoculum, keeping an inoculum-to-substrate ratio of 1.5 in terms of g VS. Regarding the ultrasound-pretreated PS, a volume of slurry corresponding to 2 g VS of original PS was subjected to AD, adding the same grams of VS from DBM used in the other BMP test as the inoculum. Demineralised water was added to reach a working volume of 150 mL, leaving 100 mL of headspace for biogas accumulation. Control, i.e., substrate-free, BMP tests were carried out to account for

the endogenous methane production. The headspace was flushed with Argon gas to ensure anaerobic conditions. The bioreactors were kept at 37 °C and regularly shaken during the incubation period. The net cumulative methane production was calculated as the average of the triplicates after subtracting the average methane production of the controls. The methane potential of raw and pretreated PS was expressed as mL CH₄/g VS. Methane production was recorded regularly until the daily accumulation was negligible in all the bottles.

The experimental results were fitted with a modified Gompertz model using Origin2018 software (OriginLab Corporation, Northampton, MA, USA) as described by Oliva et al. [12]. The correlation coefficient (r^2) between experimental and modelling data was estimated with Excel 2021 Software (Microsoft Corporation, Redmond, WC, USA) [12]. The kinetic parameters of methane production obtained from the model were used to evaluate the impact of the investigated pretreatments on the AD process.

2.5. Analytical Methods

Total (TS) and volatile (VS) solids of DBM and PS were determined according to the standard methods [25] using a TCN115 convection oven (Argo Lab, Carpi, Italy) heated at 105 °C for 24 h and a ZB1 muffle furnace (ASAL, Milan, Italy) kept at 550 °C for 2 h, respectively. The chemical composition (i.e., cellulose, hemicellulose, lignin, total extractives, and ashes content) of raw PS was determined as previously described by Oliva et al. [12].

Liquid samples were collected after pretreatment, centrifuged at 14,000 rpm for 10 min using a MIKRO 22R centrifuge (Hettich, Bremen, Germany) filtered at 0.45 µm, and stored at −20 °C for total carbohydrate and total polyphenol analysis. Total carbohydrates were determined following the phenol–sulfuric acid method [26]. Briefly, 1 mL of filtered sample, 0.5 mL of 5% phenol solution, and 2.5 mL of sulfuric acid were mixed into glass cuvettes and heated at 100 °C for 20 min. The cuvettes were left to cool down in the dark for 30 min before reading the absorbance at 492 nm using a photoLab 7600 UV-VIS spectrophotometer (WTW, Weilheim, Germany). The standard curve (0–200 mg/L) was built using glucose as the standard. Total polyphenols were measured according to the Folin–Ciocalteu (F-C) method [27]. In brief, 0.3 mL of filtered sample was mixed with 1.2 mL of 7.5% sodium carbonate and 1.5 mL of F-C reagent in quartz cuvettes. After 50 min in the dark, the absorbance was measured at 655 nm using a V-530 UV/Vis spectrophotometer (Jasco, Tokyo, Japan). The standard curve (0–250 mg/L) was built using phenol crystals as the standard.

The biogas accumulation in the headspace of the serum bottles was monitored using an LEO 1 manometer (Keller, Winterthur, Switzerland). The headspace was sampled and then released to atmospheric pressure after each sampling. The gaseous sample (5 mL) was analysed with an HPR-20 RD mass spectrometer (Hiden Analytical, Warrington, UK) equipped with a capillary tube heated at 140 °C. The liquid phase of the AD process was sampled regularly for volatile fatty acids (VFAs) analysis. The samples were centrifuged as described in the previous paragraph, filtered at 0.22 µm, and stored at −20 °C before being analysed. VFA analysis was performed using the same methodology and equipment described by Moscariello et al. [28]. The overall VFAs concentration was expressed as equivalent acetic acid per litre.

2.6. Statistical Comparison

Carbohydrate and polyphenol solubilisation, as well as methane production among the experimental conditions, were compared using the Minitab Statistical Software (Minitab LCC, Chicago, IL, USA). The difference was considered statistically significant when one-way analysis of variance (ANOVA) followed by the Tukey post hoc test returned a *p*-value lower than 0.05. The results of the statistical analyses are reported in the supporting information accompanying this manuscript (Figures S1–S5).

3. Results and Discussion

3.1. Characteristics and Potential of Raw Peanut Shells

The VS content (Table 3) of PS (i.e., 97% of TS) suggests a great potential for biofuel production, with VS being considered an indicator of the biodegradable matter content [29]. On the other hand, the lower VS content (Table 3) of the inoculum (i.e., 69% of TS) indicates that, apart from serving as the source of microorganisms, DBM is rich in minerals that can provide micro- and macronutrients necessary to enhance the AD process [30,31]. Additionally, digestate from animal manure is an extra source of nitrogen to balance the carbon/nitrogen ratio in AD of LWs [31].

Table 3. Chemical characteristics of the peanut shells and inoculum.

Parameter	Peanut Shells	Inoculum
TS (%) ^a	96.14 ± 0.07	7.80 ± 0.05
VS (%) ^a	93.63 ± 0.09	5.38 ± 0.05
VS/TS (g/g)	0.97	0.69
Total extractives (%) ^b	9.21 ± 0.12	-
Cellulose (%) ^{b, c}	28.66 ± 0.41	-
Hemicellulose (%) ^{b, d}	11.50 ± 0.20	-
Lignin (%) ^{b, e}	35.10 ± 0.54	-

^a Based on wet mass. ^b Based on dry mass. ^c Cellulose content was considered equal to glucan content. ^d Hemicellulose content was calculated as the sum of xylan, mannan, arabinan, galactan, and rhamnan sugars. ^e Lignin content was obtained as the sum of acid-soluble and acid-insoluble lignin.

Despite VS being a good indicator of biodegradable matter, not all VS are biodegradable, especially for complex substrates such as LWs [12]. Therefore, PS was further characterised to assess the cellulose, hemicellulose, and lignin content (Table 3). First, all non-bound matter, i.e., the extractives, were removed to avoid misinterpretation of the structural components' content. The extractives include all components soluble in water and 95% ethanol [32]. The total extractive content of PS (i.e., 9.21%) is in line with the values reported in the literature for other LWs, such as rice straw (7.8% [33]) and pinewood (12.4% [34]). On the other hand, other nut residues, e.g., hazelnut skin (27.5% [12]) and non-edible seeds (up to 50.6% [35]), are significantly richer in extractives. The extractives are generally associated with biodegradable matter, e.g., soluble proteins, lipids, and non-bound sugars, but also substances that can inhibit the AD process, such as polyphenols [14].

The compositional analysis (Table 3) revealed the high sugar content of PS (i.e., 40.16%), being mainly associated with glucan (i.e., 28.66%) and xylan (i.e., 10.08%). Glucan is a hexosan generally well correlated with the cellulose content of LWs, whereas xylan (pentosan) is one of the hemicellulose sugars (e.g., xylan, mannan, arabinan, galactan, and rhamnan) [36]. In particular, xylan accounted for 94% of the hemicellulose sugars in PS (supporting information, Figure S6). Xylan is the dominant hemicellulose sugar in forest and agricultural residues [37]. On the other hand, other unconventional LWs such as spent coffee grounds are rich in mannan [12]. Despite the sugar content suggesting high biodegradability, the high lignin content, i.e., 35.10% (Table 3) and the compact external surface can limit the conversion of PS to methane [38], indicating that performing pretreatments can enhance the bioavailability of the fermentable sugars present in PS.

3.2. Screening of Biomolecule Solubilisation Using Different Ultrasonic Devices

Figure 1 shows that carbohydrate solubilisation was higher ($p < 0.05$) when using the probe for ultrasonic wave diffusion regardless of the E_d . The type of device had less impact on polyphenol solubilisation. Nevertheless, statistical analyses (Supplementary Materials) showed that the ultrasonic probe was more effective ($p < 0.05$), also on polyphenol release. The maximal carbohydrate release was 40.55 mg/g TS, achieved with the ultrasonic probe and applying an E_d of 11,622 kJ/kg VS (Figure 1). Nevertheless, the statistical comparison revealed no significant difference ($p > 0.05$) among the E_d tested, indicating the smaller E_d ,

i.e., 5811 kJ/kg VS, was sufficient to solubilise the maximal carbohydrate percentage under the experimental conditions applied in this study.

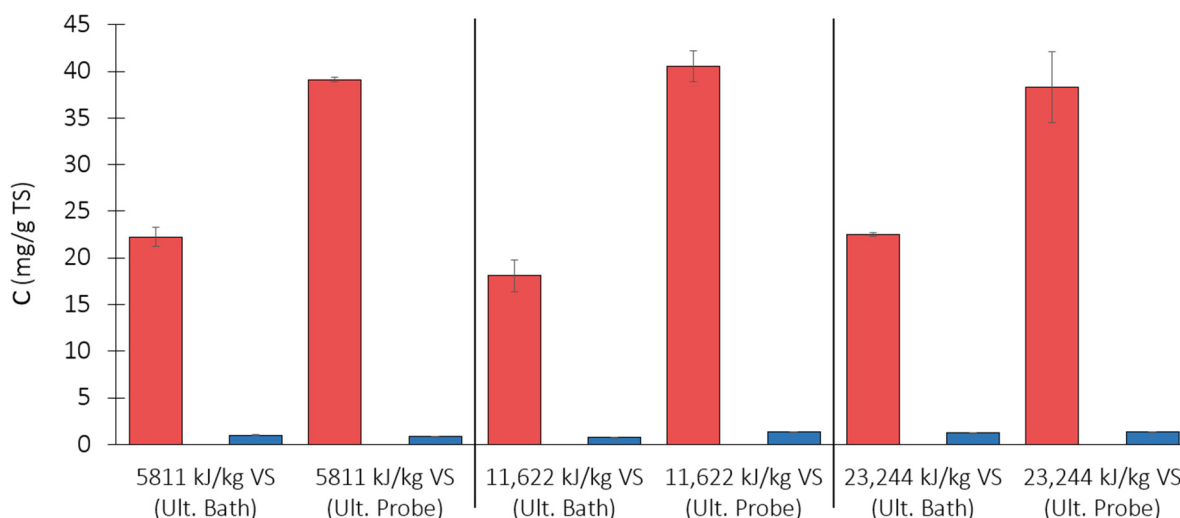


Figure 1. Carbohydrate (■) and polyphenol (■) solubilisation from peanut shells after ultrasound pretreatment using ultrasonic bath vs. ultrasonic probe.

Comparing carbohydrate solubilisation (approx. 4% of TS) with the overall structural carbohydrate content of PS (approx. 40% of TS) (Table 3), only 10% of the available carbohydrate was released due to ultrasounds. It is necessary to observe that glucose was used as the standard for total carbohydrate determination, whereas, based on the lignocellulosic composition (Table 3), glucose and xylan are the main expected sugars present in the liquid phase after pretreatment. Therefore, misinterpretation of the overall released carbohydrate can occur with the Dubois method. Despite this, it is clear that the solid residues of PS still possess great potential for bioconversion into valuable bioproducts. Therefore, the slurry (solid and liquid fraction) from ultrasounds pretreatment was subjected to AD in the subsequent biomethanation tests.

The effectiveness of the probe against the bath can be attributed to the difference in ultrasonic wave diffusion. Indeed, the intensity of ultrasounds is reduced when passing through the medium (i.e., distilled water) and the glass vessel, resulting in lower energy transferred to the biomass [39]. On the other hand, the probe was placed in direct contact with PS since it was immersed in the glass container. Similarly to the tests using the probe, no significant ($p > 0.05$) effect of the E_d was observed on carbohydrate release from PS when using the ultrasonic bath (Figure 1).

Polyphenols released during pretreatment are generally associated with lignin disruption [40]. Some nut residues, such as hazelnut skin, are particularly rich in free polyphenols, i.e., up to 106 mg gallic acid equivalent (GAE)/g TS, accounting for total extractives in the chemical composition [41]. In these cases, high polyphenol release can be observed with little correlation with the actual lignin breakdown. Compared to hazelnut skin, the polyphenol content in PS extractives is negligible (i.e., 428–740 μg GAE/g TS [42]). Therefore, the polyphenols measured in the liquid fraction after pretreatment can be fairly correlated with lignin removal. Despite that the polyphenol concentration being higher when using the ultrasonic probe ($p < 0.05$), the overall poor polyphenol release indicates that ultrasounds were not effective on the lignin component but mainly affected the holocellulose of PS, regardless of the applied E_d .

3.3. Organosolv vs. Ultrasound Pretreatment for Biomolecule Solubilisation from Peanut Shells

The carbohydrate and polyphenol analysis conducted on the liquid fraction recovered after pretreatment suggests that organosolv pretreatment is more effective than ultrasounds for lignin disruption, resulting in significantly ($p < 0.05$) higher polyphenol solubilisation

(Figure 2). Increasing the substrate-to-solvent ratio during organosolv pretreatment from 1:5 to 1:10 resulted in a higher ($p < 0.05$) polyphenol solubilisation (i.e., from 3.7 to 4.7 mg/g TS) (Figure 2), whereas the ratio of 1:20 did not result in further significant enhancements ($p > 0.05$). Up to 29.8 mg carbohydrates per gram TS of PS was released in the solvent due to the organosolv pretreatment, with no significant impact on the substrate-to-solvent ratio ($p > 0.05$). Indeed, lignin is the most difficult component to separate from LWs; therefore, more solvent was required to achieve the maximal polyphenol removal from PS [13]. On the other hand, sugars were easily removed due to weaker bonds with the other components. The substrate-to-solvent ratio has been pointed out as the key aspect to use organosolv pretreatment in full-scale applications [43]. In this study, 1:10 was the most effective ratio to maximise the solubilisation of polyphenols and carbohydrates while minimising solvent costs. A substrate-to-solvent ratio of 1:20 would result in more solvent required for the process and further dilution of the recovered biomolecules in the slurry. Substrate-to-solvent ratios between 1:10 and 1:15 are dominant in the literature [44,45]. Nevertheless, recent studies demonstrate that this value needs to be optimised for different LWs depending on the specific characteristics of the substrate and pretreatment conditions [46].

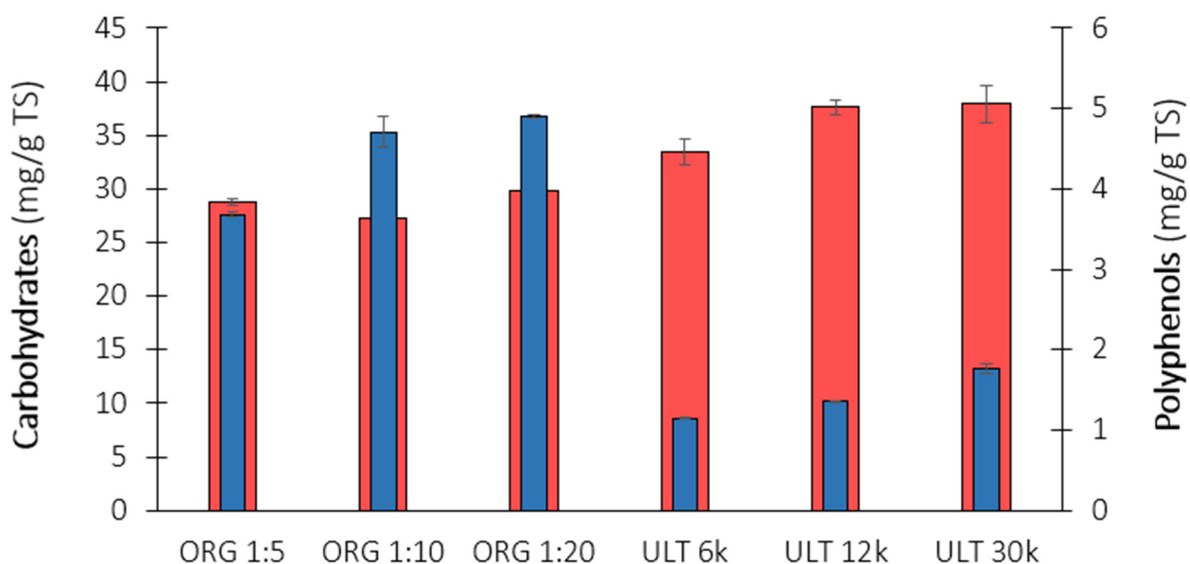


Figure 2. Carbohydrate (■) and polyphenol (■) solubilisation from peanut shells due to organosolv and ultrasounds pretreatment.

Ultrasound pretreatment mainly resulted in carbohydrate solubilisation (i.e., up to 37.9 mg/g TS of PS), with polyphenols being a minor component of the liquid fraction (Figure 2). Statistical analyses confirmed that an E_d of 12,000 kJ/kg VS is required to maximise sugar solubilisation from PS with ultrasounds pretreatment. Additionally, increasing the E_d resulted in higher ($p < 0.05$) polyphenol release. Recently, Das and Mohanty [47] combined ethanol–organosolv and ultrasound pretreatment to achieve up to 65% delignification of bamboo, opening the possibility of combining these two pretreatments. Methanol–organosolv pretreatment assisted by ultrasounds was shown effective on polyphenol release from different LWs, but no significant enhancement of the methane potential of these substrates was observed [48]. The liquid fraction from organosolv pretreatment is not suitable for direct AD due to the high methanol concentration that can hinder the AD process [48]. In addition, the presence of polyphenols can limit methane production. On the other hand, the high polyphenol and carbohydrate concentrations suggest great potential for biomolecule recovery from the organosolv liquid fraction, polyphenols being much-sought bioproducts for their antioxidant and antiviral capacity [49]. On the contrary, the whole slurry from the ultrasounds pretreatment is suitable for AD, since carbohydrates are the main component.

3.4. Methane Production and Volatile Fatty Acid Accumulation from Raw and Pretreated Peanut Shells

The methane potential of the solid residues of PS recovered after both pretreatments were compared with the raw substrate. A modified Gompertz model was used to fit the experimental data and obtain the kinetic parameters of the AD process (Table 4, Figure 3), i.e., maximum specific methane potential (G_m), maximum specific methane production rate (R_m), and lag phase (λ). The r^2 between experimental and model data ranged from 0.9880 and 0.9968, depending on the pretreatment condition (Figure 3). The strong correlation (Table 4) confirmed that the modified Gompertz here used is suitable to represent the methane production from PS in BMP tests [50]. The observed methane production trend reflects the typical behaviour of recalcitrant substrates, e.g., LWs, which shows an initial lag phase needed for microorganisms to hydrolyse cellulose and hemicellulose sugars. After that, an exponential phase in which microorganisms convert sugars into volatile fatty acids and then methane was observed. Finally, a plateau in methane production was observed when all the biodegradable matter was converted into methane [12,20].

Table 4. Kinetic parameters obtained by modelling the experimental data with the modified Gompertz model. G_m and R_m are the maximum specific methane potential and rate predicted by the model, respectively. λ is the lag phase time. r^2 is the correlation coefficient between experimental and modelling data.

Pretreatment Condition	Modified Gompertz Parameters			
	$G = G_m \times \exp \{-\exp [(R_m \times e/G_m) \times (\lambda - t) + 1]\}$			
	G_m (mL CH ₄ /g VS)	R_m (mL CH ₄ /g VS·d)	λ (d)	r^2
PS (raw)	48.08 ± 0.87	2.70 ± 0.17	4.06 ± 0.57	0.9895
PS (ORG 1:5)	40.04 ± 0.97	2.33 ± 0.19	6.40 ± 0.68	0.9880
PS (ORG 1:10)	56.12 ± 0.70	3.00 ± 0.13	5.63 ± 0.41	0.9953
PS (ORG 1:20)	45.20 ± 0.73	2.45 ± 0.13	5.99 ± 0.45	0.9940
PS (ULT 30 k)	87.52 ± 2.09	3.35 ± 0.13	5.29 ± 0.39	0.9968
PS (ULT 12 k)	64.85 ± 1.09	3.51 ± 0.22	4.40 ± 0.57	0.9894
PS (ULT 6 k)	70.37 ± 1.39	3.42 ± 0.20	4.40 ± 0.57	0.9920

None of the organosolv conditions resulted in higher methane production (Figure 4A), probably due to the loss of fermentable sugars in the liquid fraction that was not entirely compensated by lignin removal. The methane production from raw PS (i.e., 48.0 mL CH₄/g VS) was comparable ($p > 0.05$) with the organosolv-pretreated PS when using a substrate-to-solvent ratio of 1:10 and 1:20. On the other hand, when the ratio was lowered to 1:5, the methane production was significantly ($p < 0.05$) lower, i.e., 39.0 mL CH₄/g VS (Figure 4A). The model prediction (Table 4, Figure 3) showed that the G_m of organosolv-pretreated PS was slightly higher than the raw substrate when using a substrate-to-solvent ratio of 1:10, whereas it was lower or comparable when the ratio was 1:5 and 1:20, respectively (Table 4, Figure 3). The R_m reflected the trend of G_m , reaching 3.00 mL CH₄/g VS·d for the organosolv-pretreated PS at best. In contrast, λ increased in organosolv-pretreated PS, indicating that some easily biodegradable compounds may have been lost in the liquid fraction during pretreatment [51].

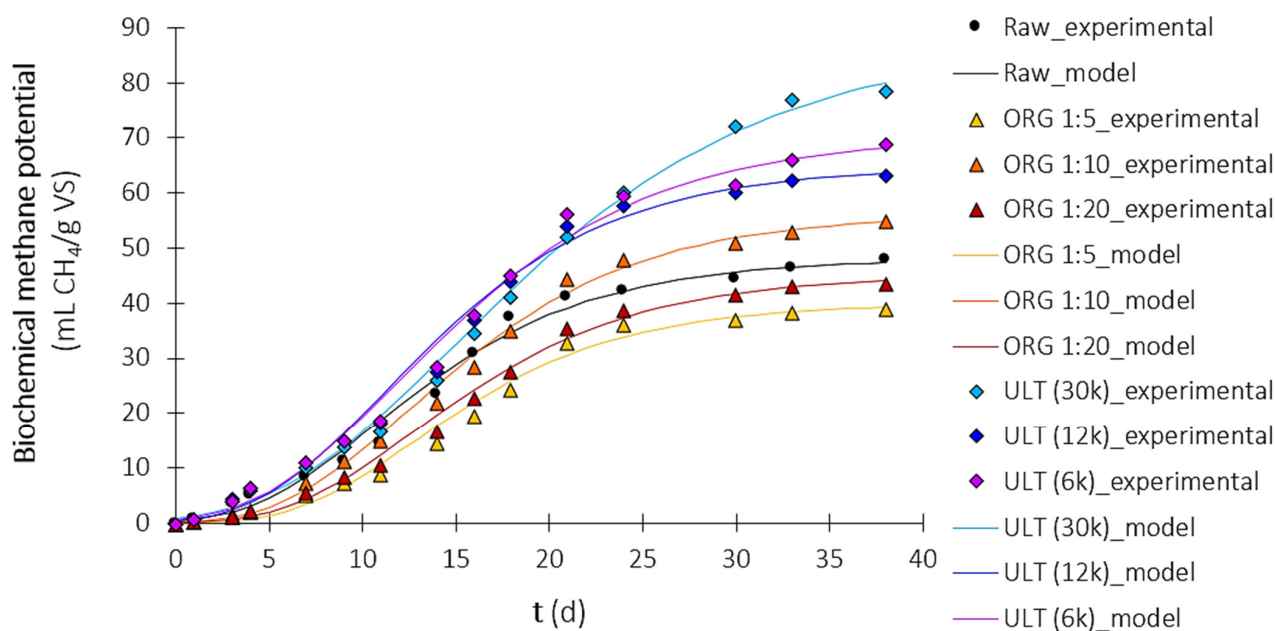


Figure 3. Fitting of the experimental methane production data with the modified Gompertz model.

Methane production can be correlated with the biomolecule release described in Section 3.3. To the best of the authors' knowledge, this is the first study investigating the impact of substrate-to-solvent ratio in the organosolv pretreatment of LWs. The solubilisation of carbohydrates regardless of the substrate-to-solvent ratio can be attributed to the effect of the temperature during the pretreatment, i.e., 130 °C, being in the appropriate range for cellulose and hemicellulose sugar release [21]. On the other hand, more solvent per gram of PS was necessary to fully enter the external surface of PS and increase lignin disruption into polyphenolic compounds. The limited loss of polyphenols when the substrate-to-solvent ratio was 1:5 suggests a lower lignin removal from PS [48], which seems insufficient to compensate for the loss of sugars. Nevertheless, BMP tests of the other organosolv-pretreated PS (i.e., 1:10 and 1:20) indicate that biomolecules can be separated from PS while keeping the methane potential untouched. Similarly to PS, methanol-organosolv pretreatment was effective on hemicellulose sugars and lignin from hemp hurds [52]. Contrary to PS, organosolv pretreatment enhanced the BMP of other nut residues, such as hazelnut skin, whereas it was ineffective on almond shells [51].

Ultrasound pretreatment significantly ($p < 0.05$) enhanced the methane potential of PS (Figure 3B). In particular, the highest E_d resulted in the maximal methane production, i.e., 78.6 mL CH₄/g VS, corresponding to 64% extra methane compared to the raw substrate. Moreover, the BMP test showed that the lowest E_d of 6000 kJ/kg VS was sufficient to improve methane production by 43%. The increment in methane potential after ultrasound pretreatment was confirmed by the G_m obtained from model fitting (Table 4, Figure 3). The increment in methane production can be attributed to the carbohydrate release that was easily available for microorganisms once solubilised in the slurry undergoing AD [53]. During AD of LWs, hemicellulose is the easily biodegradable component, whereas cellulose is more recalcitrant due to its high degree of polymerisation and crystallinity [13]. In this study, the main extra methane production was observed after several days of AD (Figure 4B). From day 18, the methane production rate from raw PS lowered while remaining higher for ultrasound-pretreated PS (Figure 4B). The R_m provided by the model reached 3.51 mL CH₄/g VS·d, at best, being 30% higher than what was observed for the raw PS, i.e., 2.70 mL CH₄/g VS·d. Interestingly, despite the increment in G_m and R_m , the ultrasound-pretreated PS showed a longer λ compared to raw PS, regardless of the E_d . This may be attributed to the release of inhibiting compounds formed during the degradation of lignin and hemicellulose due to ultrasounds, which likely lowered the AD performance in the

first few days of observation [54]. Overall, the increment in methane production after ultrasounds pretreatment can be attributed to the weakness of the bonds among the three main components of PS, i.e., cellulose, hemicellulose, and lignin, therefore resulting in easier biodegradation of the substrate [55]. Previously, the methane potential of brewery-spent grain [56] and cannabis straw [57] was shown to be enhanced using an ultrasonic probe. On the other hand, ultrasound pretreatment was not effective on the methane potential of the solid fraction of spent coffee grounds [48].

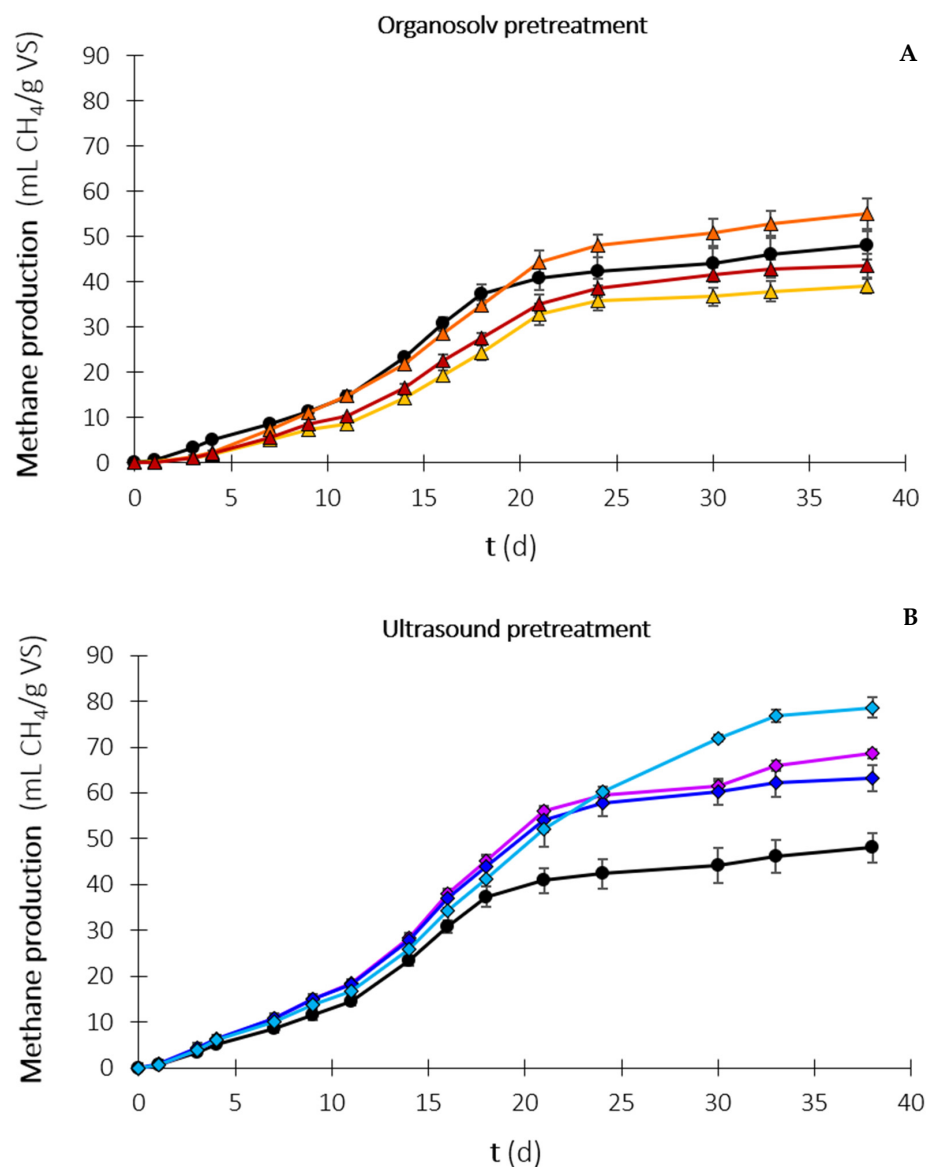


Figure 4. Cumulative methane production from anaerobic digestion of raw (●), organosolv-pretreated (A), i.e., substrate-to-solvent ratio of 1:5 (▲), 1:10 (△), and 1:20 (▲), and ultrasound-pretreated (B), i.e., applied energy density of 30,000 (◆), 12,000 (◆), and 6000 (◆) kJ/kg VS, peanut shells.

VFA accumulation during AD (Figure 5) reflected the methane production trend. VFA evolution followed the typical evolution occurring in AD of LWs [58], peaking between days 4 and 9 and then decreasing to zero. Overall, the VFA concentration was lower when feeding organosolv-pretreated PS (Figure 5C–E), whereas in ultrasound-pretreated BMP tests (Figure 5F–H) VFA accumulation was similar to that observed with the raw substrate (Figure 5B). The most abundant VFA was acetic acid, which is recognised as the main precursor for methane production via acetotrophic methanogenesis [59]. The maximal acetic acid accumulation (i.e., approx. 600 mg/L) was observed in raw and ultrasound-

pretreated conditions. Despite the higher methane production observed when subjecting PS to the highest E_d (i.e., 30,000 kJ/kg VS), no increment in VFA accumulation was observed compared to the other ultrasound conditions. In this study, acetic acid accounted for 75–93% of the total VFA production, depending on the pretreatment condition and day of AD.

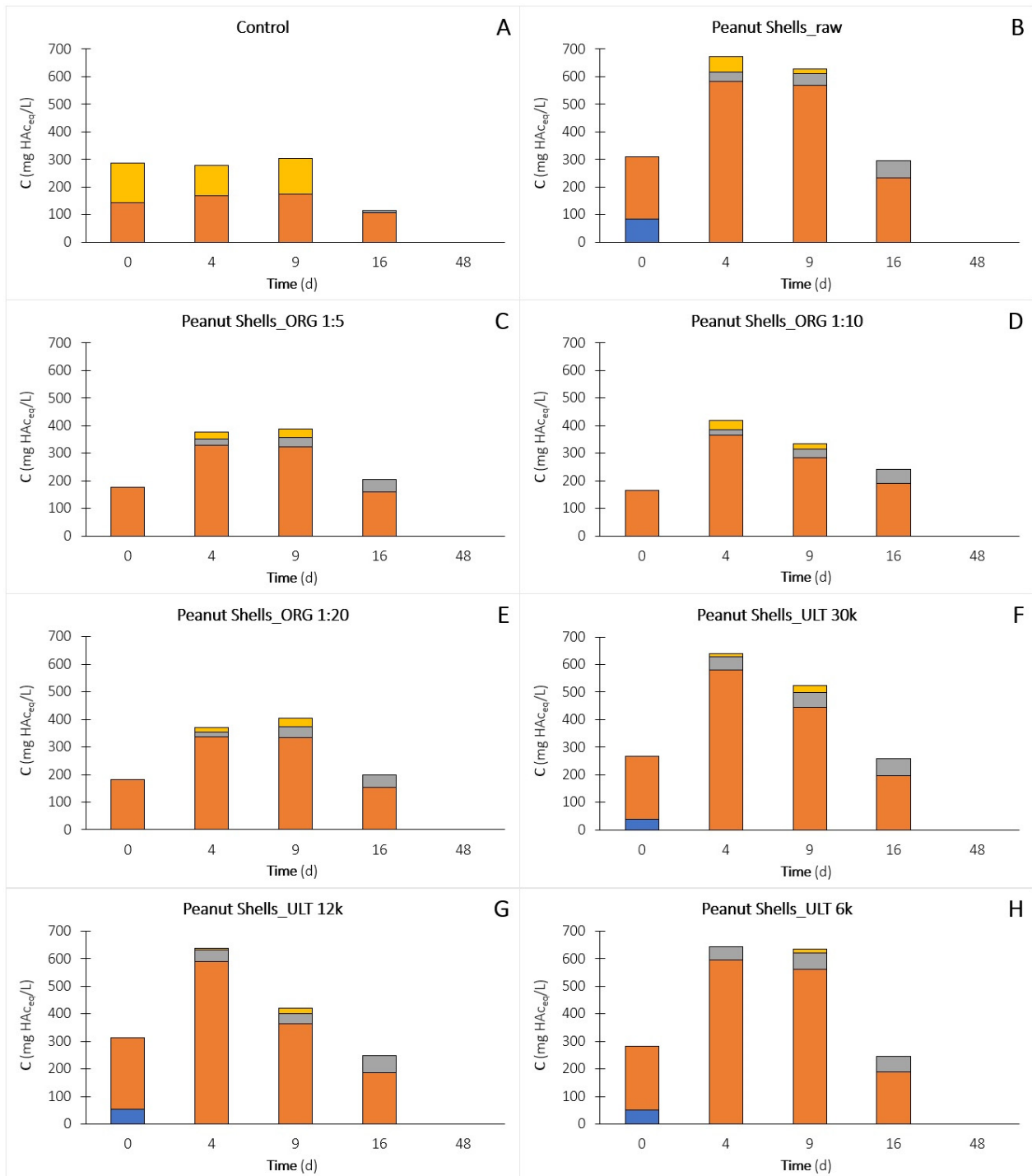


Figure 5. Volatile fatty acids accumulation (i.e., formic acid (■), acetic acid (■), propionic acid (■), and isovaleric acid (■)) during the anaerobic digestion process: control (A), raw peanut shells (B), organosolv-pretreated peanut shells using a substrate-to-solvent ratio of 1:5 (C), 1:10 (D), and 1:20 (E), ultrasound-pretreated peanut shells applying an energy density of 30,000 (F), 12,000 (G), and 6000 (H) kJ/kg VS.

Little formic acid (up to 84.40 mg HAc_{eq}/L) was detected in untreated (Figure 5B) and ultrasound-pretreated (Figure 5F–H) conditions only at the beginning of the BMP tests, indicating that formic acid was likely present in the structure of PS and was immediately released when in contact with an aqueous solution and consumed by microorganisms. Similarly, Wang et al. found that formic acid was present in the soluble fraction of another LW, i.e., rice straw [60]. On the other hand, formic acid was probably lost in the liquid fraction of the organosolv pretreatment not undergoing AD. The concentration of propionic acid (up to 62.64 mg HAc_{eq}/L) measured from day 4 was constantly well below the inhibitory threshold value suggested for AD processes [61]. Finally, the traces of isovaleric acid detected in this study can be attributed to the DBM since it was also present in the control tests at day zero (Figure 5A). The presence of isovaleric acid in the inoculum can be attributed to an incomplete AD of the buffalo manure from which the DBM was obtained. Indeed, isovaleric acid is a complex organic acid that may not be completely degraded in AD when the process is unstable [62]. Nevertheless, after 16 days of AD, the leftover isovaleric acid was completely degraded under all the experimental conditions tested in this study, including the control (Figure 5).

4. Conclusions

Organosolv and ultrasounds are effective pretreatment techniques to enhance the valorisation of PS via high-value bioproduct recovery and energy production from generated methane. Organosolv allowed releasing the highest number of polyphenols, i.e., up to 4.9 mg/g TS, from PS due to lignin disruption. In particular, the substrate-to-solvent ratio of 1:10 allowed maximal polyphenol solubilisation while maintaining the methane potential of the solid residue recovered after pretreatment similar to that of the raw PS, i.e., 55.0 mL CH₄/g VS. The liquid fraction is an attractive product that can be designated to downstream processes focusing on the recovery of the solvent, polyphenols, and sugars. In particular, the recovery of the solvent and the separation of biomolecules from the broth represent the main costs to account for when upscaling the organosolv pretreatment. Ultrasounds enabled an increase in methane production from PS, achieving 78.6 mL CH₄/g VS when increasing the applied E_d. The main effect of ultrasound pretreatment was observed on carbohydrate solubilisation, reaching 37.9 mg/g TS, whereas the number of released polyphenols was significantly lower than that achieved with organosolv. Therefore, ultrasound pretreatment appears more suitable for direct AD and subsequent energy recovery from the methane produced. In this view, the main operational cost of such pretreatment is the energy required to generate the ultrasonic waves. Thus, an optimization of the E_d applied is necessary depending on the specific substrate involved.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/en16124698/s1>, Figures S1–S6: statistical analyses.

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