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Accumulation of Volatile Fatty Acids from Hydrothermally Treated Strawberry Extrudate through Anaerobic Fermentation at Different pH Values

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Abstract: The accumulation of volatile fatty acids (VFAs) through the anaerobic fermentation of organic waste, such as strawberry extrudate, is proposed for this work. A hydrothermal treatment was carried out, and it was proposed to break the complex matrix of the strawberry extrudate to favour the hydrolysis stage of the anaerobic digestion process. The production of volatile fatty acids from treated and untreated strawberry was evaluated by adjusting the pH to 5 and 9. After the hydrothermal treatment of the strawberry extrudate, an increase in the solubilisation of organic matter, such as sugars and phenols, was observed. In the production of VFAs by means of anaerobic digestion of the pretreated strawberry extrudate, a significant increase in the accumulation of volatile fatty acids was demonstrated at a pH of 9 with respect to the untreated strawberry extrudate. In addition, the operational pH also had a strong effect on the individual VFA profile. A stream enriched in acetic acid was obtained at a pH of 9 (around 65% of the VFAs), whereas the operation at a pH of 5 resulted in a more complex composition with a high percentage of propionic acid (29% of the VFAs).

Keywords: lignocellulosic residue; pretreatment; acetic acid; agrowastes; biorefinery

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1. Introduction

The valorisation of organic waste as a substrate for biorefinery processes is part of the efforts to move from an economy based on fossil fuels to a greener circular economy based on the use of renewable sources [1]. This allows a paradigm shift in the agricultural sector where organic waste becomes a potential source of benefits, such as energy or chemicals, instead of waste to be treated at the lowest possible cost [2,3]. Agricultural waste has been widely proposed as a substrate for biorefinery processes due to its availability, low cost, and, in some cases, high content in sugars, which are highly desirable for different biological processes [2].

Strawberry extrudate (SE) is an organic substrate generated during the extrusion of the strawberries for the elaboration of a mash destined for the production of strawberry-tasted products [4,5]. SE is mainly composed of the lignocellulosic fibres and achenes of the strawberry, retained in the sieves during the extrusion process. Moreover, SE also presents a high content of valuable compounds, such as sugars and phenolic compounds, which

would be of high interest for different biorefinery processes [5]. However, the utilisation of a lignocellulosic material such as SE as a substrate for biorefinery processes would be limited due to the necessity of hydrolysing the biomass to yield fermentable sugars or the presence of microbial inhibitors, such as phenolic compounds [6,7].

Previous studies have proposed the use of pH-controlled anaerobic fermentation as a suitable method for the hydrolysation of organic substrates and their transformation into valuable building blocks, mainly volatile fatty acids (VFAs) [8–11]. In fact, the economic value of VFAs is usually much higher than the economic value of the methane, typically obtained from anaerobic digestion processes [12]. Actually, methane has a market value of only EUR 16.63–33.27/mt [13], whereas acetic acid and propionic acid have values of around EUR 499/mt and EUR 3546/mt, respectively [14]. The anaerobic reactor of the pH-controlled anaerobic fermentation operates at pH values other than 7–8 that is recommended for methanogenic activity [15], avoiding the conversion of the solubilised compounds into biogas and, thus, favouring the accumulation of VFAs. Therefore, higher hydrolysis rates and VFA concentrations could be obtained under acidic or alkaline conditions in comparison with conventional anaerobic fermentation at a neutral pH. For example, Liu et al. [11] reported a hydrolysis rate of 68.7% for the alkaline fermentation of sewage sludge at a pH of 10 in a full-scale plant, reaching a VFA yield of 261.32 mg COD/g VSS (COD, chemical oxygen demand; VSS, volatile suspended solid). However, the hydrolysis is more limited for substrates with a high content in lignocellulosic fibres. For example, Cabrera et al. [8] described that the hydrolysis of olive mill solid waste reached values of 50% and 56% at an operational pH of 5 and 9, respectively, whereas the operation at a pH of 7 allowed the biomethanisation of more than 90% of the added substrate. In the same line, a previous study by Cubero-Cardoso et al. [16] concluded that the lignocellulosic structures of SE would hamper the action of the hydrolytic enzymes, limiting the solubilisation of the substrates and the accumulation of VFAs. Indeed, these authors reported hydrolysis rates of 55% and 50% at an operational pH of 5 and pH of 9, respectively, for the anaerobic fermentation of SE at semibatch conditions. Despite the limitation on the hydrolysis rates, the operation at an acidic or alkaline pH allowed an effective inhibition of the methanogenesis and the conversion of most of the soluble organic matter into VFAs [16].

Due to the limited solubilisation reported for SE, it would be interesting to evaluate a pretreatment method that would facilitate the hydrolysis of the substrate and, thus, improve the process yield. The implementation of hydrothermal treatments has been widely proposed for enhancing the hydrolysis of lignocellulosic substrates [6,17]. The application of high temperature and pressure conditions aims to partially solubilise the cellulose and hemicellulose and, at the most severe conditions, also affect the lignin structure [17]. Previous studies have studied the solubilisation of SE through hydrothermal treatments at a wide range of operational conditions [5,18,19]. Rodríguez-Gutiérrez et al. [18] evaluated the solubilisation of SE at the temperature range from 90 °C to 200 °C, concluding that the application of temperatures around 150 °C for one hour maximised the solubilisation of the lignocellulosic fibres into simpler compounds, mainly sugars. The same authors also stated the necessity of carrying out a purification step before further biological treatment of the hydrothermally treated substrate for removing the antioxidant and antimicrobial products that would act as microbial inhibitors [18]. However, the conversion of the solubilised compounds during the hydrothermal treatments to a constant flow enriched in VFAs is still required for favouring the valorisation of SE in biorefinery processes.

Therefore, the aim of the present research is the evaluation of a hydrothermal pretreatment to the pH-controlled anaerobic fermentation of SE at a pH of 5 and 9 for maximising the accumulation of VFAs. For that, the process will be compared with the accumulation of VFAs of the pH-controlled anaerobic fermentations without the pretreatment stages. The processes will be evaluated according to the solubilisation capacity for the substrate, as well as the composition of the accumulated compounds at each operational condition and the further effect on the microbial activity at the pH-controlled anaerobic fermentations.

2. Materials and Methods

2.1. Inoculum and Substrates Used during the Experimentation

The inoculum used for seeding the anaerobic reactors was obtained from a full-scale mesophilic anaerobic reactor located in an urban wastewater treatment plant located in Seville, Spain. The inoculum presented a total solid (TS) concentration of 21.3 g/kg, a VS/TS ratio of 0.63, and a pH of 7.12.

The raw substrate for the experimental procedure, i.e., the strawberry extrudate (SE), was provided by the company Hudisa S.A., located in Lepe, Spain. The SE was kept at −18 °C to avoid undesirable fermentation processes prior to the experimentation. A fraction of the SE was subjected to a hydrothermal treatment to evaluate a possible enhancement of the hydrolysis of the substrate. In particular, 12.6 kg of raw SE was subjected to a hydrothermal treatment (150 °C, 60 min, 5 kg/cm^2), generating the hydrothermally treated strawberry extrudate (HTSE) used during the experimentation.

2.2. Experimental Set-Up and Experimental Design

pH-controlled anaerobic fermentations of the SE and the HTSE were evaluated in triplicate at a pH of 5 and 9. Anaerobic reactors with 1.7 L working volume (2.0 L total volume) were used. The anaerobic reactors were continuously stirred through a magnetic bar and kept in a thermostatic chamber at 35 ± 1 °C. The operational pH of the reactors was monitored daily and corrected, if necessary, by adding HCl (12 N) or NaOH (3 N). SE and HTSE were batch-fed once per week for a total period of 50 days. Biogas production was monitored daily throughout the experimental time. Samples of the mixing liquor of each reactor were taken and analysed five times per week to evaluate the pH-controlled anaerobic fermentations of the SE and the HTSE at both a pH of 5 and 9.

2.3. Analytical Methods

The determinations of the total solids (TSs), total fixed solids (FSs), total volatile solids (VSs), total chemical oxygen demand (COD_{tot}), and soluble chemical oxygen demand (COD_s) were carried out by following the methodologies established by American Public Health et al. [20]. For measuring soluble compounds, the samples were filtered by means of 0.45 µm pore filters. The total and soluble phenolic compound concentrations were determined through the Folin–Ciocalteu method (more details in [21]). Total and soluble sugar concentrations were measured through the anthrone colorimetric method [19]. The concentration of hydroxymethylfurfural was quantified using a Hewlett-Packard 1100 liquid chromatography system. The individual volatile fatty acids (VFAs), i.e., C2–C5, were determined using a Shimadzu gas chromatograph (GC-2010). Individual VFA concentrations were expressed as mg O_2/L. More details of the equipment and analysis conditions for the determination of the hydroxymethylfurfural and individual VFA can be found in Serrano et al. [22].

2.4. Calculations

The evaluation of the metabolic activity at each experimental condition was carried out by the determination of the hydrolysis, acidogenesis, and methanogenesis activities based on the conversion of the added substrate according to the following expressions (Equations (1)–(3)):

$$\text{Hydrolysis} = \frac{\text{CODs} + \text{COD}_{\text{CH}_4}}{\text{COD}_{\text{added}}} \quad (1)$$

$$\text{Acidogenesis} = \frac{\text{COD}_{\text{VFA}} + \text{COD}_{\text{CH}_4}}{\text{COD}_{\text{added}}} \quad (2)$$

$$\text{Methanogenesis} = \frac{\text{COD}_{\text{CH}_4}}{\text{COD}_{\text{added}}} \quad (3)$$

where COD_s was the concentration of soluble COD in the reactor; COD_{CH4} was the methane production expressed as g COD, and COD_{VFA} was the sum of the individual volatile fatty

acids expressed as g COD. COD_{added} was the accumulated substrate added to each reactor at each time and expressed as g COD.

Sigmaplot 11.0 and Microsoft Excel 2016 were used for the calculation of the mean and standard deviation values of the triplicate data, as well as for the elaboration of the figures.

3. Results

3.1. Effect of the Hydrothermal Treatment in Organic Matter Solubilisation

The implementation of the proposed hydrothermal treatment at 150 °C for one hour had a strong effect on the soluble parameters of hydrothermally treated strawberry extrudate (HTSE) compared to strawberry extrudate (SE). The soluble sugars and the soluble phenols in HTSE increased around ten-fold compared to SE (Table 1). Similarly, soluble chemical oxygen demand (COD_s) increased after the pretreatment step, varying from 47 ± 1 g O_2/g SE to 61 ± 1 g O_2/g SE (Table 1). The solubilisation effect of the proposed pretreatment can be corroborated by the increase in the COD_s/COD_{tot} ratio in HTSE compared to SE, which presented values of 33% and 23%, respectively. The hydrothermal treatments at temperatures around 150 °C applied to lignocellulosic substrates, as the SE, mainly solubilised the hemicelluloses and, to a lesser extent, part of the lignin structures [17,23]. In HTSE, a concentration of up to 505 ± 1 mg/kg SE of hydroxymethylfurfural was detected (Table 1). The formation of hydroxymethylfurfural, a well-known inhibitor of anaerobic digestion, is a side effect of the application of hydrothermal treatments to lignocellulosic substrates [24]. However, the concentration of hydroxymethylfurfural in HTSE was in a range where no obvious negative effects are usually detected according to different authors [24–26].

Table 1. Analytical characterisation of the strawberry extrudate and the hydrothermally treated strawberry extrudate.

	SE	HTSE
pH	3.7 ± 0.1	4.1 ± 0.1
Moisture (%)	85.5	87.0
TSs (g/kg SE)	145 ± 4	130 ± 2
FSs (g/kg SE)	5 ± 1	6 ± 2
VSs (g/kg SE)	139 ± 4	124 ± 3
COD_{tot} (g O_2/kg SE)	200 ± 6	186 ± 3
COD_S (g O_2/kg SE)	47 ± 1	61 ± 1
%Ratio (COD_S/COD_{tot})	23	33
Total Phenols (mg gallic acid/kg SE)	2185 ± 64	1751 ± 62
Soluble Phenols (mg gallic acid/kg SE)	103 ± 2	987 ± 34
Total Sugars (mg glucose/kg SE)	$29{,}466 \pm 431$	$26{,}799 \pm 1227$
Soluble Sugars (mg glucose/kg VS)	2023 ± 99	$19{,}591 \pm 721$
Hydroxymethylfurfural (mg/g SE)	<D.L.	505 ± 1

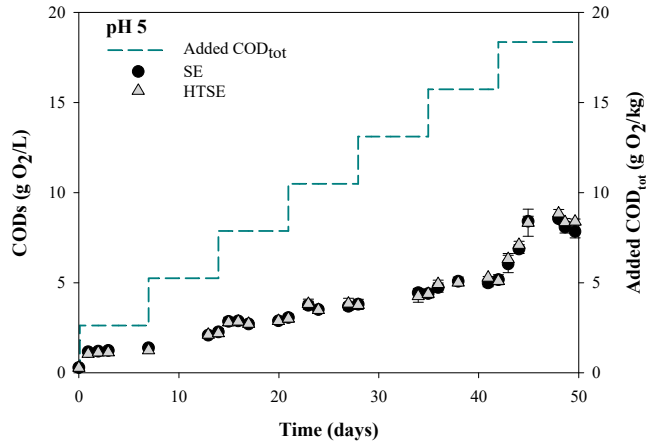
SE, strawberry extrudate; HTSE, hydrothermally treated strawberry extrudate; D.L., detection limit; TSs, total solids; FSs, Fixed solids; VSs, volatile solids; COD_{tot}, total chemical oxygen demand; and COD_s, soluble chemical oxygen demand.

The total organic matter concentration, measured as total volatile solid (VS) and total chemical oxygen demand (COD_{tot}), indicated that most of the organic matter in the SE remained in HTSE after the hydrothermal treatment (Table 1). The slight decrease in these parameters, around 10% of the initial organic matter, would result from the release of some organic volatile compounds due to steam condensation [27]. On another hand, an increment in the soluble phenol concentration was observed for HTSE compared to SE (Table 1), indicating that part of the phenolic compounds presented in the substrate were solubilised after the hydrothermal treatment.

3.2. Solubilisation of the Substrate and Methane Production

The implementation of the hydrothermal treatment process did not entail a significant effect at an operational pH of 5, which presented, at the end of the experimental time, COD_s

concentrations for SE and HTSE of 8.9 ± 0.2 g O_2/L and 8.4 ± 0.3 g O_2/L, respectively (Figure 1a). These values are close to those reported by Serrano et al. [10], which achieved a COD_s concentration of around 9 g O_2/L during the anaerobic fermentation of wasted berries at a pH of 4. Otherwise, operation at a pH of 9 resulted in a slight decrease in the COD_s for HTSE compared to SE, i.e., 6.4 ± 0.2 g O_2/L and 5.8 ± 0.2 g O_2/L, respectively (Figure 1b). Regardless of the difference in the COD_s concentration, the anaerobic fermentation at a pH of 5 and 9 resulted in a similar COD_s to added COD ratio, with values of 47 ± 2% and 45 ± 4%, respectively (Figure 1a,b). The lack of a solubilisation effect deriving from the hydrothermal treatment is due to the experimental conditions, i.e., 150 °C for one hour, which were not severe enough to allow the degradation of the most recalcitrant compounds in SE, such as the lignin presented in the achenes [4,17]. Because the rest of the strawberry fruit is readily biodegradable, the proposed pH values of 5 and 9 were enough for COD solubilisation without the necessity of a previous pretreatment.



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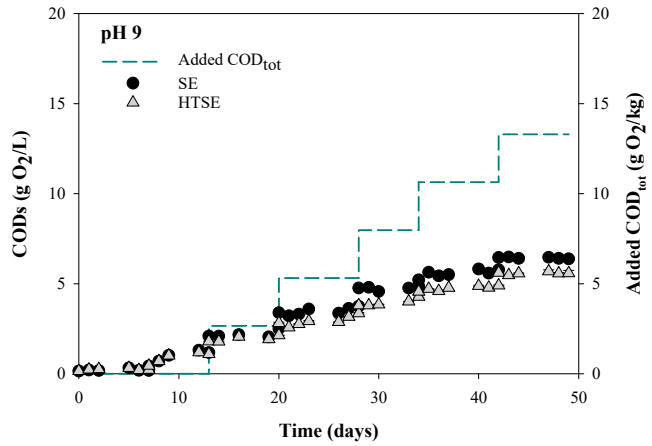


(**b**)

Figure 1. Solubilisation of SE and HTSE organic matter in COD (**a**) at a pH of 5 and (**b**) at a pH of 9 with respect to feed.

At a pH of 5, the accumulated methane production was not totally inhibited, reaching a value of around 1.8 g O_2/g VS for both SE and HTSE (Figure 2a). This accumulated methane production represented around 10% of the added COD_{tot} to the reactors. On the other hand, the inhibition of the methane production for favouring the volatile fatty acid (VFA) accumulation was more effective at an operational pH of 9, where the accumulated methane production was undetectable for both SE and HTSE (Figure 2b). The inhibition of the methane production at acidic and alkaline pH values was previously described by several authors [10,28]. This inhibition occurs due to the operation at a pH out of the optimal

range for the methanogenic activity, i.e., 7.3–7.8, but where the bacteria still have hydrolytic and acidogenic activities [28,29]. The total inhibition of methane production at a pH of 9 (Figure 2b) is contradictory to the results reported by Serrano et al. [10] for the anaerobic fermentation of wasted berries as the phenol solubilisation method, which reached a value of up to 215 ± 10 mL CH_4/g VS. The difference in the methane production would be explained by the lack of correct pH control in Serrano et al. [10], because these authors reported a drop in the pH to values around 7 while the methane production occurred.

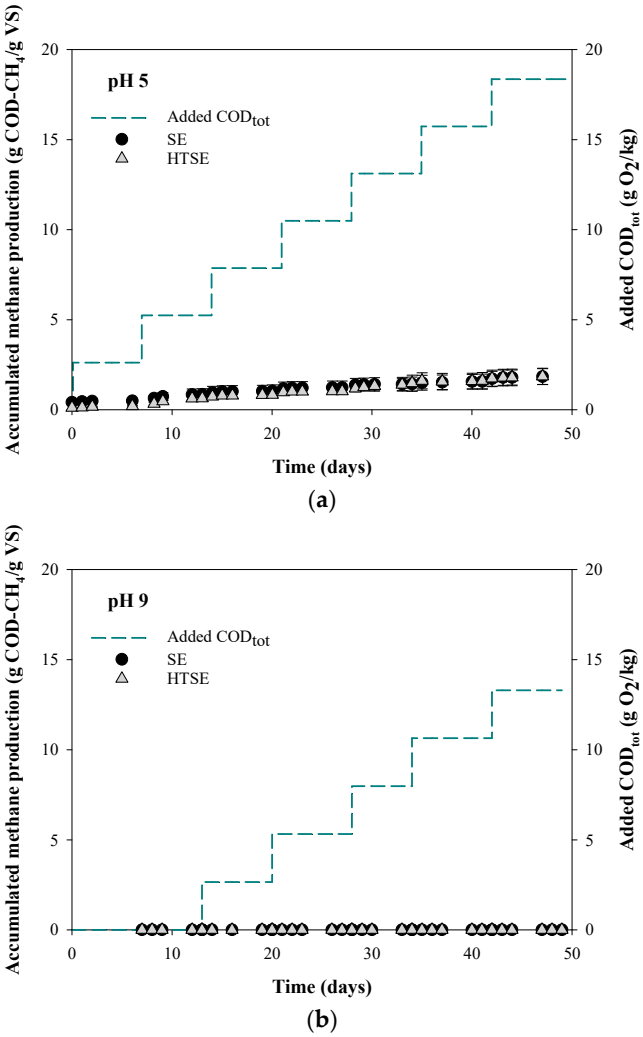
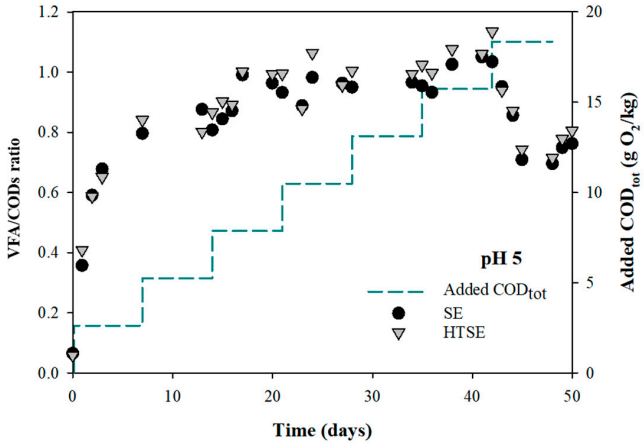


Figure 2. Accumulated methane production from SE and HTSE (**a**) at a pH of 5 and (**b**) at a pH of 9 with respect to feed.

3.3. Accumulation of Volatile Fatty Acids

The sum of the individual VFA concentrations, expressed in grams of COD, were very similar at a pH of 5 for SE and HTSE at the end of the experimental time, i.e., 6.1 ± 0.4 g O_2/L and 6.5 ± 0.2, respectively. Similarly, the proposed pretreatment stage did not show any effect in the fermentation at a pH of 9, resulting in VFA concentrations of 5.5 ± 0.5 g O_2/L and 5.6 ± 0.5 for SE and HTSE, respectively. Most of the soluble compounds were in the form of VFAs according to the VFA/COD_s ratio, regardless of the operational pH and pretreatment (Figure 3a,b). The highest conversion of soluble compounds to VFAs was achieved at a pH of 5, where the VFA/COD_s ratio values were close to 1 throughout most of the experimental time. It is worth noting that a difference in the VFA/COD_s ratios was observed at a pH of 9 between SE and HTSE after 25 days of operation, HTSE being markedly higher than SE (Figure 3b). Indeed, HTSE presented a mean VFA/COD_s

ratio of 0.93 ± 0.08, whereas SE resulted in a mean VFA/COD_s ratio of 0.80 ± 0.08. This difference would indicate that the proposed hydrothermal treatment would remove some recalcitrant compounds that were not possible to convert into VFAs at an operational pH of 9, such as soluble dietary fibres or phenolic glucosides, which can be hard to metabolise by microorganisms [18,30]. Despite the similar effectiveness in the conversion of the soluble compounds into VFAs at both operational pH values, the operation at a pH of 9 with and without pretreatment resulted in a much higher conversion of the added COD_{tot} into VFAs than the operation at a pH of 5, i.e., 42 ± 1 % and 34 ± 2 %, respectively. These results were in line with those reported by other authors, which described that the alkaline conditions would favour the activity of some enzymes involved in the degradation of carbohydrates and proteins [31,32].



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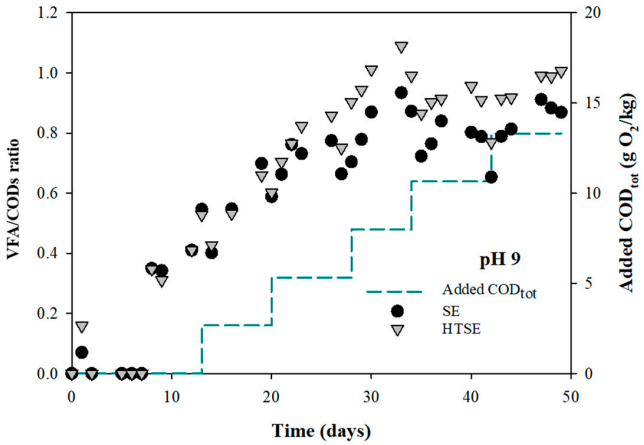
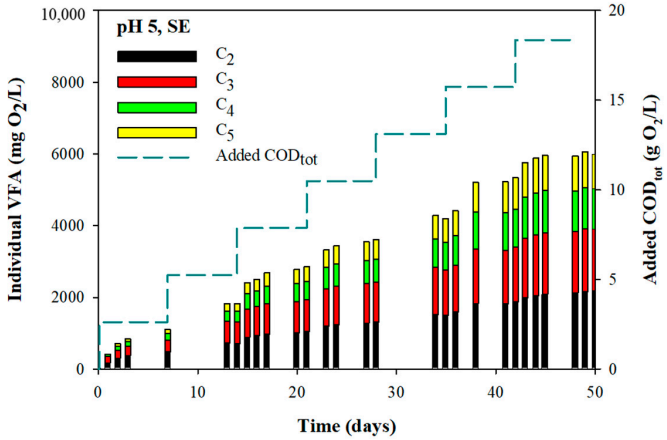


(**b**)

Figure 3. Relation from VFAs to COD_s from SE and HTSE (**a**) at a pH of 5 and (**b**) at a pH of 9 with respect to feed.

The operational pH also had a strong effect on the individual VFA profile, with strong differences between the concentrations of each VFA determined at a pH of 5 and 9 (Figure 4a–d). On the opposite side, the implementation of the hydrothermal treatment did not result in remarkable differences between SE and HTSE at both an operational pH of 5 (Figure 4a,b) and pH of 9 (Figure 4c,d). Acetic and propionic acids were the predominant VFAs at a pH of 5, representing around 35% and 29% of the sum of the VFAs, respectively. Butyric and valeric acids were presented in a lesser, but meaningful percentage, reaching concentrations around 1200 mg O_2/L and percentages of 19% and 17% of the sum of the VFAs, respectively (Figure 4a,b). The individual VFA profile for SE and HTSE at a pH of

9 showed that acetic acid was the predominant accumulated VFA, representing around 65% of the sum of the VFAs (Figure 4c,d). The other individual VFA compounds reached similar low concentration values, around 700 mg O_2/L, which represented percentages of the sum of the VFAs around 10–15%. The higher preponderance of acetic acid at an alkaline operational pH was previously described by several authors [10,33,34]. The almost total inhibition of methanogenesis at an alkaline pH would favour the accumulation of acetic acid, instead of its conversion into methane [33].



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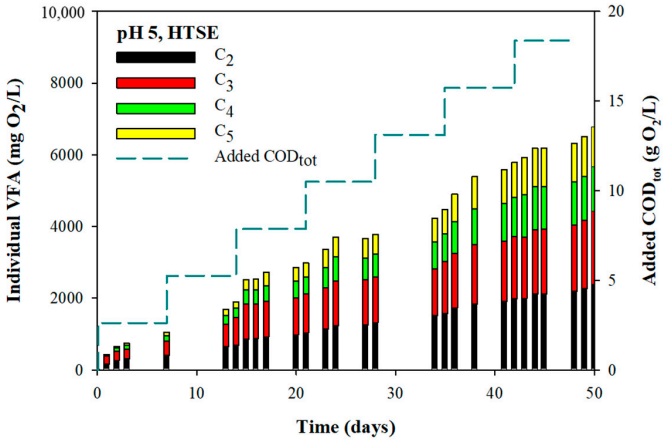

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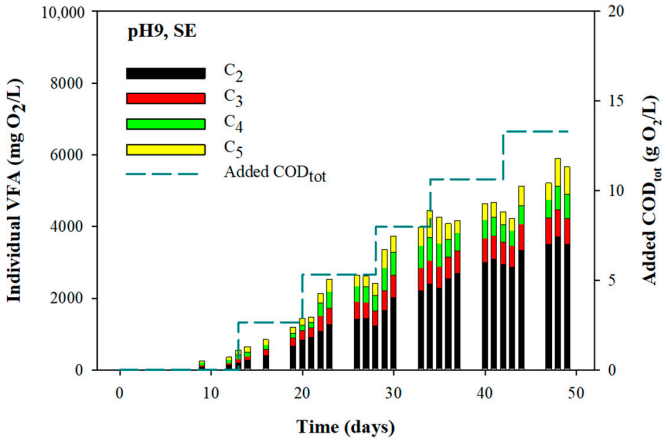
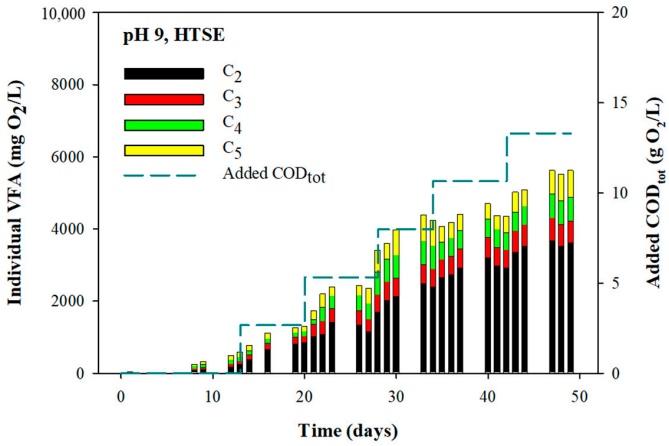


(**c**)

Figure 4. *Cont.*



(**d**)

Figure 4. Individual VFA compounds, i.e., acetic acid, propionic acid, butyric acid, and valeric acid accumulated for (**a**) pH5 SE, (**b**) pH5 HTSE, (**c**) pH 9 SE, and (**d**) pH9 HTSE against the accumulated added substrate (added COD_{tot}) throughout the experimental time.

3.4. Concentration of Soluble Sugars and Soluble Phenols

The concentration of soluble sugars at the different operational conditions varied in a low range below 100 mg glucose/L during most of the experimental time (Table 2). The avoided accumulation of soluble sugars was in line with the high effectiveness in the conversion of COD_s into VFAs described in the previous Section 3.3. The concentration of soluble phenolic compounds throughout the experimental time showed that the operation at a pH of 5 resulted in a higher accumulation of phenolic compounds compared to the operation at a pH of 9 (Table 2). The concentration of soluble phenols achieved values around 150 mg gallic acid/L at a pH of 5, whereas a pH of 9 only reached values around 100 mg gallic acid/L. A similar difference was previously described by Serrano et al. [10] for the evaluation of the fermentation of wasted strawberries as a method for the solubilisation of phenols. These authors described that the operation at a pH of 4 favoured the accumulation of phenolic compounds compared to the operation at a neutral or an alkaline pH, reaching values between 300 and 400 mg gallic acid/L. Despite the accumulation of phenolic compounds in the reactors during the anaerobic fermentation, the obtained values were markedly lower than those determined in the substrate (Table 1), indicating that phenolic compounds were degraded to simpler compounds during the anaerobic fermentation process. The anaerobic degradation of the phenolic compounds has been widely reported as long as the concentrations were far from the inhibition range for these compounds [34–36].

Table 2. Concentrations of soluble sugars (mg glucose/L) and soluble phenols (mg gallic acid/L) at different experimental times.

Days	pH 5			
	Soluble Sugars (mg Glucose/L)		Soluble Phenols (mg Gallic Acid/L)	
	SE	HTSE	SE	HTSE
0	48 ± 5	43 ± 7	99 ± 3	90 ± 2
7	41 ± 2	38 ± 5	170 ± 7	152 ± 23
21	19 ± 1	19 ± 2	119 ± 6	112 ± 2
28	66 ± 9	63 ± 3	107 ± 5	101 ± 3
35	59 ± 6	57 ± 4	166 ± 6	162 ± 5
43	49 ± 5	49 ± 2	149 ± 10	141 ± 23

Table 2. *Cont.*

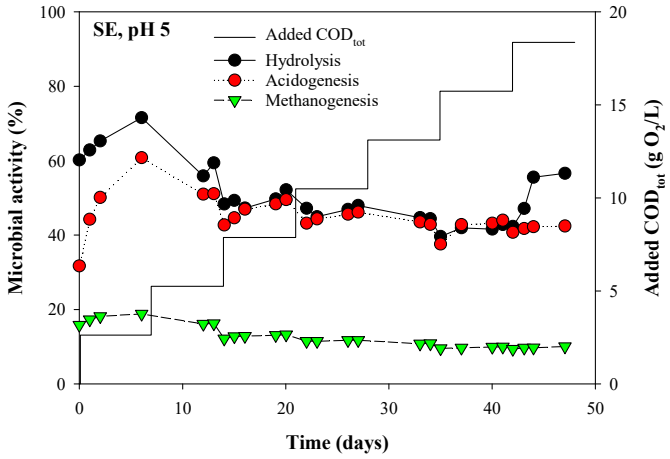
Days	pH 9			
	Soluble Sugars (mg Glucose/L)		Soluble Phenols (mg Gallic Acid/L)	
	SE	HTSE	SE	HTSE
0	-	-	19 ± 7	23 ± 5
13	157 ± 136	152 ± 142	58 ± 3	53 ± 7
20	51 ± 9	47 ± 5	67 ± 5	65 ± 9
28	57 ± 13	53 ± 2	72 ± 15	69 ± 17
34	63 ± 10	63 ± 4	102 ± 15	100 ± 31
42	62 ± 9	89 ± 2	95 ± 21	86 ± 25
49	65 ± 5	89 ± 7	89 ± 31	106 ± 31

SE, strawberry extrudate; HTSE, hydrothermally treated strawberry extrudate.

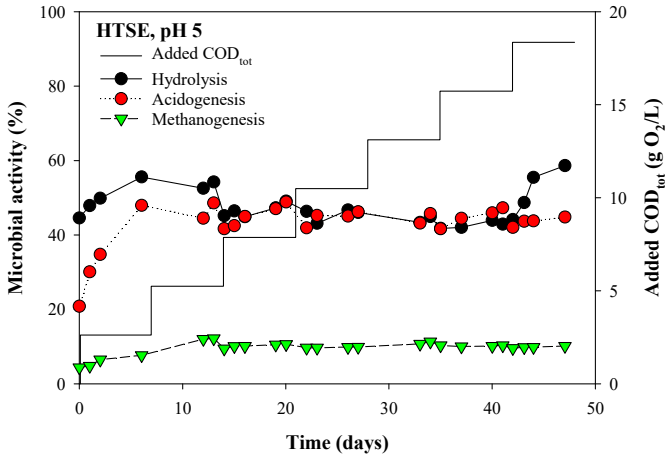
3.5. Microbial Metabolic Activity Stages

The hydrolysis and acidogenesis activities showed almost constant values during the operation at a pH of 5 for both SE and HTSE after the 7-day operation (Figure 5a,b). Concurrently, the hydrolysis and the acidogenesis presented mean values of 47.5 ± 5.0% and 44.6 ± 2.8%, respectively, indicating that the conversion of the solubilised compounds during the hydrolysis were rapidly converted into VFAs. This was an expected behaviour because hydrolysis is usually defined as the rate-limiting step in the anaerobic degradation of organic solids [37]. The most significant difference between the reactors treating SE and HTSE occurred during the first 10 days of operation, where the methanogenic activity for SE was 17.5 ± 1.3%, whereas for HTSE it was markedly lower, i.e., 5.8 ± 1.5% (Figure 5a,b). After this initial period, both SE and HTSE presented a similar methanogenic activity with a mean value of 10.8 ± 1.4% (Figure 5a,b). This methanogenesis activity entailed that a fraction of the generated VFAs were consumed. Enhancing the accumulation of VFAs would be interesting in future research on the implementation of more extreme pH values; e.g., the total inhibition of the methanogenesis has been described at a pH of 4 [10], decreasing the retention time for diminishing the acetogen populations due to their longer growth time compared to the other involved fermentative bacteria, or varying other operational parameters, such as the temperature or the organic loading rate [38,39].

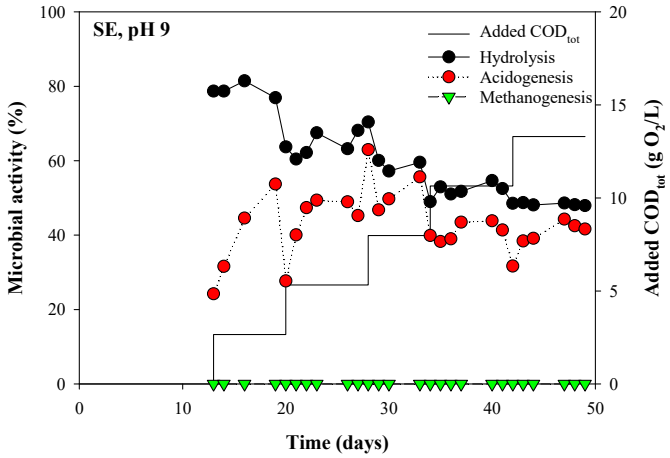
At an operational pH of 9, the microbial metabolic activities showed a different trend compared to that described for the operation at a pH of 5. For SE at a pH of 9, the hydrolytic activity decreased from values around 80% to a stable value of 48.4 ± 0.3% at the end of the experimental time (days 42 to 49) (Figure 5c). However, the anaerobic fermentation of HTSE at a pH of 9 resulted in a lower hydrolytic activity, with values varying from around 60% at the start of the experiment to a stable value of 36.5 ± 0.4% at the end of the experimental time (days 42 to 49) (Figure 5d). Despite the differences in the hydrolysis activity, the anaerobic fermentation at a pH of 9 of SE and HTSE showed similar acidogenic activity, with mean values of 39.6 ± 4.5% and 34.0 ± 3.3%, respectively, at the end of the experimental time (days 42 to 49) (Figure 5c,d). The methanogenic activity was totally inhibited for both SE and HTSE, avoiding the conversion of VFAs into methane and allowing the accumulation of acetic acid [38]. The stronger inhibition of the methanogenic activity at alkaline conditions in comparison to acidic conditions was previously described by different authors [40,41]. At the same time, the lower hydrolysis and acidogenesis described at a pH of 9 in comparison to a pH of 5 was compensated by the stronger inhibition of the methanogenic activity, explaining the similar VFA accumulation capacities for both operational pH values. Therefore, the selection of the operational pH should be considered in relation to the desirable target VFA compounds to be obtained. Otherwise, the implementation of hydrothermal treatments could be avoided considering the observed low effect on the VFA accumulation. Further research would be interesting to evaluate more severe treatments that would allow the solubilisation of the lignin fraction of the SE, evaluating if the expected increase in VFAs is enough to compensate for the generation of potential inhibitors and the higher energy requirements.



(**a**)

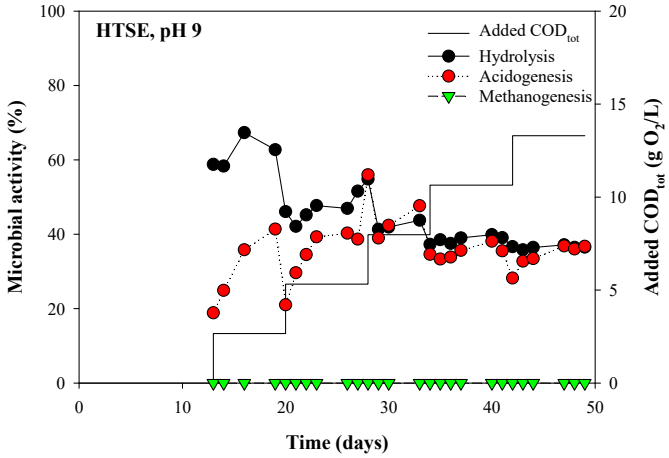


(**b**)



(**c**)

Figure 5. *Cont.*



(**d**)

Figure 5. Relation of the stages of anaerobic digestion processes at a pH of 5 from (**a**) SE and (**b**) HTSE and at a pH of 9 from (**c**) SE and (**d**) HTSE.

4. Conclusions

This study successfully demonstrated the accumulation of volatile fatty acids (VFAs) from hydrothermally treated and untreated strawberry extrudate (SE) by anaerobic digestion, showing an increased solubilisation after hydrothermal treatment. Methanogenic activity was almost inhibited at both evaluated pH values despite the hydrothermal treatment. The extent of VFA accumulation was, however, strongly dependent on the pH conditions. The total VFA concentrations of 6.5 ± 0.2 and 5.6 ± 0.5 g O_2/L were obtained at a pH of 5 and 9, respectively, after hydrothermal treatment from SE. Furthermore, it was demonstrated that the relation between the operational pH and the obtained individual VFA profile obtained a more complex VFA composition at a pH of 5.

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Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

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