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Volatile Fatty Acids (VFA) Production from Wastewaters with High Salinity—Influence of pH, Salinity and Reactor Configuration

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Abstract: The hydrocarbon-based economy is moving at a large pace to a decarbonized sustainable bioeconomy based on biorefining all types of secondary carbohydrate-based raw materials. In this work, 50 g L⁻¹ in COD of a mixture of food waste, brine and wastewater derived from a biodiesel production facility were used to produce organic acids, important building-blocks for a biobased industry. High salinity (12–18 g L⁻¹), different reactors configuration operated in batch mode, and different initial pH were tested. In experiment I, a batch stirred reactor (BSR) at atmospheric pressure and a granular sludge bed column (GSBC) were tested with an initial pH of 5. In the end of the experiment, the acidification yield (ηa) was similar in both reactors (22–24%, w/w); nevertheless, lactic acid was in lower concentrations in BSR (6.3 g L⁻¹ in COD), when compared to GSBC (8.0 g L⁻¹ in COD), and valeric was the dominant acid, reaching 17.3% (w/w) in the BSR. In experiment II, the BSR and a pressurized batch stirred reactor (PBSR, operated at 6 bar) were tested with initial pH 7. The ηa and the VFA concentration were higher in the BSR (46%, 22.8 g L⁻¹ in COD) than in the PBSR (41%, 20.3 g/L in COD), and longer chain acids were more predominant in BSR (24.4% butyric, 6.7% valeric, and 6.2% caproic acids) than in PBSR (23.2%, 6.2%, and 4.2%, respectively). The results show that initial pH of 7 allows achieving higher ηa, and the BSR presents the most suitable reactor among tested configurations to produce VFA from wastes/wastewaters with high salinity.

Keywords: VFA; high-salinity wastewater; biorefineries; food waste; anaerobic digestion

1. Introduction

Biogas is the primary product obtained in anaerobic digestion (AD) processes that present the double advantage of stabilizing biodegradable waste materials and producing renewable energy. Currently, AD processes are being explored to obtain other products, such as organic acids, that may be used as building-blocks for chemicals and biofuels, replacing the need for fossil fuel-based products. These present a higher market value when compared with the energy production from biogas [1].

The production of organic acids is achieved by stopping the process at an early AD stage—acidogenesis. Salinity and pH are two important operating variables that can drive the process, resulting in the interruption or continuation of steps, such as the accumulation of VFA or production of biogas [2]. Usually, low pH inhibits the methanogenic activity [3], thereby favoring the accumulation of by-products of the initial stages of AD, including VFA [4]. On the other hand, if pH levels are near neutrality, there is no hindrance to the methanogenic activity, and VFA can be further converted into biogas [5]. The same happens by regulating the levels of salinity [6]. The presence of salt in wastewater is a
concern that can result from the use of seawater for toilet flushing, or from seawater intrusion in coastal WWTP due to the increase in sea-level and flood tide events [7,8]. These events can impact salinity to levels up to 8–19 g L⁻¹ [8]. Moreover, saline wastewaters are produced daily by several industries, such as tanneries, oil and gas producing, and seafood processing (including fish processing) [9]. The anaerobic treatment of these saline wastewaters is a challenge since anaerobic microorganisms are known to be sensitive to the presence of high sodium/or chloride concentrations [10], in particular the methanogenic archaea. These microorganisms can be more sensitive than acidogenic bacteria to high salinity [11], and their activity can typically be inhibited under salt concentrations around 20 g L⁻¹ [6,12].

In addition to those parameters, the bioreactor configuration may be an important feature to address, in order to optimize VFA production from bio-wastes. Generally, the acidogenesis proceeds sufficiently fast in well mixed tanks; however, other configurations have potential to be used for VFA production, and comparisons should be performed [13,14]. For example, in expanded systems such as the expanded granular sludge blanket reactor, the high liquid velocities leads to a slight expansion of the sludge-bed, which results in an excellent contact between sludge and wastewater [13]. Another example may be the pressurized reactors. The application of simultaneous high pressure and temperature has enabled to increase between two and five times the VFA yield from sewage sludge [15], suggesting pressure as a potential variable of interest to improve the acidogenesis of complex wastes, such as food waste (FW). In this way, it may be expected that the application of pressure by using pressurized bioreactors may improve the solubilization of wastes with high solid content, such as FW, therefore facilitating its hydrolysis and further acidification.

In this work, FW from a canteen, brine from a fish-canning industry, and wastewater derived from a biodiesel production facility, with a final salinity of [12–18] g L⁻¹, were mixed and used to produce VFA in different reactor configurations/conditions (a batch stirred reactor, a pressurized stirred batch reactor, and a granular sludge bed column) and at different pH levels (5 and 7) in order to obtain the best operational conditions that promote organic acids production.

2. Materials and Methods

2.1. Experimental Set-Up and Operation Mode

This work was divided into two experiments, according to the pH tested. In experiment I, two bioreactor configurations were tested with the goal of producing VFA at an initial pH of 5: a batch stirred reactor (BSR), named BSR-5; and a granular sludge bed column (GSBC) reactor with recirculation, named GSBC-5 (Figure 1). In experiment II, an initial pH of 7 was tested in a BSR-7 and in a pressurized stirred batch reactor (PBSR-7) (Figure 1). In both experiments, the reactors were operated in batch mode, due to the high solids content in FW. In BSR-7 and PBSR-7, 5 g L⁻¹ of sodium bicarbonate was used as buffer. All reactors were operated at 37 °C.

The GSBC reactor consisted of a plexiglass cylinder with a total volume of 2.25 L and a working volume of 2 L, with external liquid recirculation that promoted the sludge bed expansion and better contact and mixing. The BSR had a total volume of 5 L and a working volume of 2.5 L, was made of stainless steel, and contained internal stirring paddles. The stainless steel PBSR had a total volume of 2 L and a working volume of 1.2 L (PARR Model 4524, Parr, Moline, IL, USA). PBSR also contained internal stirring paddles. This reactor was pressurized with N₂, reaching a final pressure of 6 bar. Samples for VFA, chemical oxygen demand (COD), pH, and salinity were collected over time. In the PBSR, the reactor was depressurized for sampling, and then re-pressurized to 6 bar with N₂.
2.2. Substrates and Inoculum

The same inoculum was used for the four reactors, and consisted of anaerobic granular sludge from a brewery wastewater treatment plant (Super Bock, Porto, Portugal). The sludge was previously boiled at 100 °C for 10 min to suppress the methanogens [16], in order to avoid the further degradation of the fatty acids produced. The sludge presented the following concentration in volatile solids (VS) in the reactors: (13.1 ± 1.8) g L⁻¹ in BSR-5; (12.7 ± 0.6) g L⁻¹ in GSBC-5; (20.8 ± 5.6) g L⁻¹ in BSR-7; and (20.8 ± 5.6) g L⁻¹ in PBSR-7.

The substrate consisted of a mixture of FW (Canteen of the University of Minho, Braga, Portugal) and wastewater (WW) derived from a biodiesel production facility, 50/50 in total COD, in a final concentration of approximately 50 g L⁻¹. The FW was homogenized by blending the solid waste in tap water. Additionally, brine from the fish-canning industry (A Poveira, Póvoa de Varzim, Portugal), was added to give high salinity to the mixture. The brine salinity was 224.3 g L⁻¹. The salinity was measured before the experiment, and was constant over time, presenting values of (12.2 ± 2.2) g L⁻¹ in GSBC-5 and (12.3 ± 1.5) g L⁻¹ in BSR-5, in experiment I. In experiment II, the salinity content was increased to (17.8 ± 1.5) g L⁻¹ in PBSR-7 and (18.4 ± 1.5) g L⁻¹ in BSR-7.

The characterization of the substrates used (FW and Biodiesel WW) regarding its content in total COD (tCOD), total solids (TS), and VS is presented in Table 1.

Table 1. Characterization of the substrates in g of parameter per kg of substrate.

|       | FW                     | Biodiesel WW    |
|-------|------------------------|----------------|
| tCOD  | 169.7 ± 19.8           | 61.4 ± 1.9     |
| TS    | 163.2 ± 5.3            | 39.3 ± 0.2     |
| VS    | 146.1 ± 6.7            | 15.2 ± 0.1     |

2.3. Analytical Methods

Samples from the reactors content (containing sludge, substrates, and products) were collected over time. The samples for VFA (acetic, propionic, iso- and n-butryric, iso- and n-valeric, and iso- and n-caproic acids), lactic acid, and soluble COD (sCOD) quantification were centrifuged for 15 min at 15000 rpm and filtered through a 0.2 μm nylon syringe filter. VFA and lactic acid were analyzed using a Jasco (Tokyo, Japan) HPLC equipped with a PU880 JASCO isocratic pump, a 7971 Jones Chromatography oven, an AS2057Plus automatic injector, and a UV-2070plus detector. The mobile phase was composed of a 2.5 mmol L⁻¹ H₂SO₄ solution. COD (total and soluble) was determined spectrophotometrically using cuvette test kits LCK 014 (Hach-Lange GmbH, Düsseldorf, Germany). Sample dilu-
tion (1:10) was previously carried out, to avoid the interference of salinity in COD quantification. A Hach Lange HT 200 S thermostat and a Hach Lange DR 2800 spectrophotometer were used in this analysis. pH was measured with a Hanna (Woonsocket, RI) pH-meter and salinity measurements were conducted using CO 301 digital conductivity meter (VWR, Radnor, PA, USA). Total and volatile solids were determined according to the Standard Methods [17]. Possible biogas production was quantified with a Ritter MilliGas counter (Dr.-Ing. Ritter Apparatebau GmbH, Bochum, Germany).

2.4. Calculations

VFA and lactic acid results are presented in this work in g L⁻¹ of COD. The theoretical COD equivalence for lactic, formic, acetic, propionic, butyric, valeric, and caproic acids were 1.07, 0.35, 1.07, 1.51, 1.82, 2.04, and 2.21 g of COD g⁻¹ of acid, respectively. These values were calculated according to Equation (1):

\[ C_nH_{2n+1}COOH + (n + \frac{a}{4} - \frac{b}{2} - \frac{3}{4}c)O_2 \rightarrow nCO_2 + \left(\frac{a}{2} - \frac{3}{2}c\right)H_2O + cNH_3 \]  

(1)

The acidification yield (\(\eta_a\)) was defined as the ratio between the total VFA + lactic acid (in COD) in the reactor and the tCOD fed to the reactors [18–20], according to Equation (2):

\[ \eta_a(%, w/w) = \frac{COD_{VFA} + COD_{lactic acid}}{tCOD} \times 100 \]  

(2)

The ratio between VFA + lactic acid (in COD) and the sCOD measured at each point, was calculated according to Equation (3):

\[ VFA/sCOD(%, w/w) = \frac{COD_{VFA} + COD_{lactic acid}}{sCOD} \times 100 \]  

(3)

The total VFA corresponds to the sum of each VFA and lactic acid expressed as g of COD.

3. Results

pH, salinity and reactor configurations were tested with the focus on optimizing the VFA production in two experiments. In experiment I BSR and GSBC were tested with an initial pH of 5 and salinity of 12 g L⁻¹. In experiment II, the pH was set to 7, salinity to 18 g L⁻¹, and the reactors evaluated were the BSR and the PBSR.

Experiment I

Figure 2 shows the VFA and pH profiles. At day two, VFA concentration attained the maximum in BSR-5, while in the GSBC-5 VFA were much lower and almost constant over the experiment (Figure 2a,b). At this point, the pH seemed to be stabilized (Figure 2c). It dropped to 3.5 in day one, and did not vary over time. Lactic acid was the main acid produced in both reactors (Figure 2a,b), presenting a maximum concentration of 6.3 g L⁻¹ in COD in the BSR-5 and 8.0 g L⁻¹ in COD in the GSBC-5. A clear difference in VFA profiles was observed in both reactors, with significantly higher concentrations of acetic (3.9 g L⁻¹ in COD), propionic (2.1 g L⁻¹ in COD), and iso-valeric (1.6 g L⁻¹ in COD) acids obtained in the BSR-5 in two days, in comparison with the GSBC-5, where these acids achieved maximum values of 0.9 g L⁻¹, 0.7 g L⁻¹, and 1.4 g L⁻¹ in COD, for acetic, propionic, and iso-valeric acids, respectively, in five days. In the GSBC-5, n-butyric acid was also one of the main VFA produced (0.9 g L⁻¹ in COD), while in BSR-5, this acid was not detected. The remaining VFA, such as formic, iso-butyric, n-valeric, iso-caproic and n-caproic acids, were not detected or their quantification was below the detection limit (<25 mg L⁻¹). In the BSR-5, the \(\eta_a\) and the VFA/sCOD ratio reached a maximum of 28% and 37%, respectively, in two days, and then decreased slightly to 22% and 30% at day five. In the GSBC-5 the \(\eta_a\) achieved a maximum of 24% (VFA/sCOD of 30%) on the last day of experiment (Table 2). In both reactors, the sCOD almost did not change over time, being 36 g L⁻¹ in the BSR-5
and 39.4 g L⁻¹ in the GSBC-5 at the end of operation (day five). Biogas production did not occur over the experiment.

**Figure 2.** VFA and lactic acid profiles in reactors GSBC-5 (a) and BSR-5 (b), over time. (c) Time course of pH in reactors GSBC-5 and BSR-5.
Table 2. Total VFA (plus lactic acid) concentration in COD (VFA), soluble COD (sCOD), ratio VFA/sCOD, and acidification yield (ηa) in the reactors, over the experiments I and II.

| Time (d) | GSBC-5 | BSR-5 | PBSR-7 | BSR-7 |
|----------|--------|-------|--------|-------|
|          | VFA (g/L in COD) | sCOD (g/L) | VFA/sCOD | ηa % | VFA (g/L in COD) | sCOD (g/L) | VFA/sCOD | ηa % | VFA (g/L in COD) | sCOD (g/L) | VFA/sCOD | ηa % | VFA (g/L in COD) | sCOD (g/L) | VFA/sCOD | ηa % |
| 0        | 0.9    | 38.4  | 3      | 2     | 1.1  | 35.9  | 3      | 2     | 6.1  | 28.6  | 21    | 12    | 6.1  | 28.6  | 21    | 12    |
| 1        | 9.1    | 40.4  | 23     | 18    | 8.2  | 37.8  | 22     | 16    | 11.9 | 30.7  | 39    | 24    | 13.5 | 33.8  | 40    | 27    |
| 2        | 9.9    | 41.2  | 24     | 20    | 14.1 | 38.5  | 37     | 28    | 10.8 | 32.2  | 34    | 22    | 16.1 | 33.3  | 48    | 32    |
| 5        | 11.9   | 39.4  | 30     | 24    | 11.0 | 36.0  | 30     | 22    | 15.0 | 36.1  | 42    | 30    | 19.6 | 36.1  | 54    | 39    |
| 7        | -      | -     | -      | -     | -    | -    | -      | -     | -    | 17.6 | 36.3  | 48    | 35    | 17.7 | 36.9  | 48    | 35    |
| 9        | -      | -     | -      | -     | -    | -    | -      | -     | -    | 18.9 | 35.9  | 53    | 38    | 20.4 | 36.5  | 56    | 41    |
| 12       | -      | -     | -      | -     | -    | -    | -      | -     | -    | 19.1 | 36.5  | 52    | 38    | 22.0 | 36.4  | 60    | 44    |
| 14       | -      | -     | -      | -     | -    | -    | -      | -     | -    | 20.3 | 37.2  | 55    | 41    | 22.8 | 34.7  | 66    | 46    |

Experiment II

Since the best VFA profile and the fastest VFA production were obtained in the BSR-5, in experiment II two stirred batch reactors were tested at an initial pH of 7. The same BSR of experiment I and a pressurized batch stirred reactor were compared. The reasoning for using a pressurized reactor was to assess if high pressure could have a positive effect on hydrolysis, and could ultimately increase the rate and the degree of acidification. The VFA and pH profiles in those reactors are presented in Figure 3.

The VFA profile was similar in both reactors, but slightly higher concentrations of the main VFA, namely propionic (8.0 g L⁻¹ in COD), acetic (6.2 g L⁻¹ in COD), and n-butyric (4.5 g L⁻¹ in COD) acids were recorded in the BSR-7, when compared to PBSR-7 (7.8 g L⁻¹, 5.7 g L⁻¹ and 4.0 g L⁻¹ in COD, respectively). In both reactors, lactic acid was present in high concentration (3.6 g L⁻¹ in COD) at the beginning of the fermentation, but after one day, it started to decrease. On the first day of operation, the iso-valeric acid concentration increased in both reactors until it reached approximately 3.6 g L⁻¹ in COD, but immediately after, on day two, the concentration decreased to 0.3 g L⁻¹ and 0.8 g L⁻¹ in COD in PBSR-7 and BSR-7, respectively (Figure 3a,b). Over the experiment, the other acids (formic, iso-butyric, n-valeric, iso-, and n-caproic) presented concentrations below 2 g L⁻¹ in COD. The pH decreased from 7 to around 6.5 on the first day, and it remained stable over time in BSR-7 (Figure 3c). In the PBSR-7, the minimum pH recorded was 6.1 on day seven, but it increased until 6.4 in the end of the experiment (Figure 3c). At the end of the experiment, the ηa and the VFA/sCOD ratio in the BSR-7 were 46% and 66%, while in the PBSR-7 they were 41% and 55% (Table 2), respectively. Both PBSR-7 and BSR-7 reactors started with the same sCOD of 28.6 g L⁻¹, which then increased to 37.2 g L⁻¹ and 34.7 g L⁻¹, in PBSR-7 and BSR-7, respectively (Table 2), at the end of experiment II. Moreover, in experiment II, biogas production was not detected.
Figure 3. VFA and lactic acid profiles in reactors PBSR-7 (a) and BSR-7 (b), over time. (c) Time course of pH in reactors PBSR-7 and BSR-7.

At the end of operation, in both experiments I and II, the granular sludge disintegrated. This may have been caused by the salinity. The presence of high concentration of monovalent cations, such as Na⁺ ions, has been described as affecting the structure of the sludge [6,21]. The monovalent cations (Na⁺) can exchange with the divalent cations (Ca^{2+} and Mg^{2+}) present in the extracellular polymeric substances structure of sludge, promoting the disintegration of the biomass structure [22].
4. Discussion

The production of carboxylic acids from wastes has been the focus of several studies, but only recently has the potential of using wastes containing high salinity to produce these acids been explored [6,23]. In this work, the potential of alternative reactors configuration to produce VFA from wastes/wastewaters containing high salinity was assessed under initial acidic and neutral pH. First, the performance of a BSR and a GSBC was tested with an initial pH of 5, since an acidic pH is known to inhibit the methanogens [3] and is suitable for the acidogenic bacteria [24]. In a second experiment, a BSR was tested at atmospheric pressure and at a pressure of 6 bar with an initial pH of 7.

In experiment I, lactic acid was the main product. This was also reported by other authors under similar experimental conditions, i.e., lactate-type fermentation was described as being favored at pH 4, and uncontrolled pH led to lactic acid accumulation [25]. Tang et al. [26] reported the accumulation of lactic acid in reactors operated without pH control and in the absence of buffering capacity, which further caused a pH drop to values around 3. This extremely acidic pH, resulted in hydrolysis inhibition [26], which was also observed in BSR-5 and GSBC-5, since the sCOD almost did not change over the experiment (Table 2). Slightly acidic pH was suggested to be ideal for hydrolysis, since some hydrolytic enzymes have optimum activity at pH 6 [27,28]; nevertheless, pH control is essential for avoiding the drop of pH below to VFA pKa (around 4.8), in order to prevent microbial inhibition, such as happened in experiment I. Despite of the low pH, the VFA concentration obtained in this work (−11 g L⁻¹ in COD) was much higher than the one obtained by Liu et al. [29] (4.14 ± 0.44 g L⁻¹) in reactors treating FW under acidic conditions and with a salinity of 12 g L⁻¹ (Table 3). Under the tested conditions in experiment I, BSR-5 was shown to be more suitable for acids production, since higher concentrations of VFA were achieved, namely 3.9 g L⁻¹ in COD of acetic acid, 2.1 g L⁻¹ in COD of propionic acid and 1.8 g L⁻¹ in COD of iso-valeric acid (maximum at day 2), when compared to the GSBC-5 (0.9 g L⁻¹, 0.7 g L⁻¹ and 1.4 g L⁻¹ in COD, respectively, at day 5).

Based on the results obtained in experiment I, which showed that the BSR was the best configuration, in experiment II, the BSR was set with a higher initial pH 7 (BSR-7) to avoid the microbial inhibition caused by extremely acidic pH. Neutral pH was also applied in experiment II since pH drop is intensified by the presence of higher salt levels (8 and 16 g L⁻¹) [30], and by the VFA production/accumulation (experiment I). Since neutral pH may allow the reactivation of the methanogens, in experiment II, the salinity content was increased to 18 g L⁻¹ to maintain suppression of the methanogens. Methanogens have a lower acclimation capacity to high salinity concentrations than acidogens, being strongly inhibited at NaCl concentrations above 10 g L⁻¹ [10,31] Besides methanogens, salinity above 20 g L⁻¹ can also inhibit microbial activities of non-adapted acidogens [32], therefore, 18 g L⁻¹ of salinity was the maximum tested in experiment II. Since the η<sub>p</sub> increased from 22–24% in experiment I to 41–46% in experiment II, it can be concluded that, in this study, the increase of the salinity did not inhibit the acidogenic bacteria. A pressurized reactor (PBSR-7) was also set, and the performance compared with BSR-7. The results obtained in experiment II have shown that the BSR is more efficient, when comparing the yield of acidification and the VFA/sCOD ratio obtained in this reactor (46% and 66%) with that of the PBSR (41% and 55%), under the same conditions (Table 2). Approximately more than 0.5 g L⁻¹ in COD of (each) acetic, butyric, and caproic acids were produced in the BSR-7 in comparison with the PBSR-7 (Figure 3a,b). Nevertheless, the pressure applied in PBSR-7 resulted in higher solubilization of COD, i.e., in the end of the experiment, the sCOD (Table 2) was higher in the PBSR-7 (37.2 g L⁻¹) than in BSR-7 (34.7 g L⁻¹). The application of pressure as a pre-treatment to enhance the anaerobic digestion of complex wastes has been previously tested [33]. This approach increased the waste solubilization; nevertheless, that did not improve the methane production. Those results are in agreement with the results obtained in experiment II, i.e., the superior hydrolysis observed in PBSR-7 did not result in superior acidogenesis in terms of rate, nor in terms of products distribution.
For example, Lindeboom et al. [34] had previously studied the effect of pressure on methanogens, and noticed that an increase of pressure from 1 to 3 bar led to a decrease of 30% on specific methanogenic activity. Despite piezo-sensitive and piezo-tolerant species can being able to survive up to 90 bar [34] (which is much higher than the conditions tested in this work), their activity may be compromised. This may be the case of the acidogens present in the PBSR-7. The higher hydrolysis obtained in PBSR, suggests that this reactor configuration may be interesting to apply in the AD of more complex and/or recalcitrant wastes, but its effects on methanogenic and acidigenic microorganisms should be further assessed.

Comparing the results obtained in experiment II with the work of Li et al. [35], which studied the co-fermentation of FW and waste activated sludge (WAS) under similar conditions to those tested in experiment II (Table 3); the yield of acidification was much lower (estimated in 31%) than the ones obtained in BSR-7 (46%) and in PBSR-7 (41%). Moreover, regarding VFA concentration, Li et al. [35] obtained ~12 g L⁻¹ in COD, while ~20 g L⁻¹ was obtained in both reactors in experiment II. Regarding the fermentation of only FW, with a salinity of 10 and 30 g L⁻¹, and a pH of 6, the yield of acidification attained in the experiments of He et al. [36] was around 100% (estimated). The sum of the VFA produced in that work rounded to 26 g L⁻¹ which is close to the ones obtained in BSR-7 (22.8 g L⁻¹ in COD) and PBRS-7 (20.3 g L⁻¹ in COD); nevertheless, caproate and valerate were not produced, only shorter VFA were (Table 3), while in this work these longer chain acids were produced (Figure 3, Table 3). Sarkar et al. [37] obtained better results than the ones from experiment II, i.e., under a pH of 6 and 10–20 g L⁻¹ of salinity, the acidification yields of FW were around 53–61%; nevertheless, the final VFA concentration (approximately 6 g L⁻¹) was lower than the ones obtained in this study. This may be explained by the fact that the organic load tested by Sarkar et al. [37] (15 g L⁻¹ in COD) was much lower than the one tested in this work (50 g L⁻¹ in COD).

Overall, the yields of acidification obtained with initial pH 5 (experiment I) were lower (approximately half) than those obtained under initial pH 7 (6–6.5 over the experiment, Figure 3c), which is in accordance with previous works, showing that pH 6 presents a better condition to produce high yield VFA, when compared to lower pH [28], even in the presence of higher salinity. Moreover, as described in the literature, at 7 ≥ pH ≥ 5.5 the production of longer chain VFA occurs, especially butyrate, in acidigenic bioreactors treating FW [28,38,39]. In our work, propionate (up to 8 g L⁻¹ in COD) was the main VFA produced. Propionate is an interesting product, as it serves as a food additive, and it is an intermediate in the production of other chemicals, especially polymers, pesticides, and pharmaceuticals [40]. Acetate and butyrate productions were also highly representative at an initial of pH 7 (∼28% and ∼24%, respectively). Other longer chain acids, such as valeric and caproic acids, were also produced in concentrations up to 3.6 and 1.4 g L⁻¹ (as COD) in BSR-7, and up to 3.7 and 0.9 g L⁻¹ (as COD) in PBSR-7, respectively. The VFA produced can be further recovered and purified by applying downstream processes such as electrodialysis, adsorption, reverse osmosis and nanofiltration, among others. Compiled information about this and other downstream processes for VFA recovery/purification can be found elsewhere [41].

VFA are considered as the preferred precursors for the synthesis of polyhydroxyalkanoates (PHA), also known as bioplastics [42]. Polyhydroxybutyrate (PHB) is the most common PHA; nevertheless, there is an increasing interest in other PHA, such as the copolymer 3(HB-co-HV) or 3-hydroxyvalerate (3HV), since these are more flexible and stronger with preserved biodegradability than PHB, therefore they may be used in a wider range of applications [43,44]. The production of 3HV results from the conversion of odd-carbons VFA such as valerate and propionate [42], which were the main VFA produced in experiments I and II, respectively (Table 3). Moreover, an advantage of using a fermented feedstocks that has a broad spectrum of VFA, such as acetate, propionate, butyrate, and valerate, is that it allows higher accumulation rates of PHA and also to produce polymers with high diversity of hydroxyalkanoates, containing monomers other than
3HB, such as 3HV or 3-hydroxyhexanoate (3HHx) [44,45]. Therefore, we may suggest that the VFA produced in this study, especially the ones from BSR-7, would be suitable feedstock to produce PHA with longer and diverse chains (3HV, 3HHx, and others).

Table 3. Performance of acidogenic processes using FW and food processing WW with high salinity content. (n.d.—not determined).

| Type of Substrate | Type of Reactor | pH | COD Fed (g L\(^{-1}\)) | VFA (g L\(^{-1}\)) | η\(\text{v}\) (L L\(^{-1}\)) | Acetate (g L\(^{-1}\)) | Propionate (g L\(^{-1}\)) | Butyrate (g L\(^{-1}\)) | Valerate (g L\(^{-1}\)) | Caproate (g L\(^{-1}\)) | Ref. |
|------------------|----------------|----|---------------------|-------------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|------|
| FW               | Batch          | 0  | n.d.                | 41.06 ± 0.92      | 47.6 ± 1.5       | 8.9 ± 0.4       | 26.4 ± 0.7      | -               | -               | -               |      |
| FW               | Batch         | 6  | 200                 | n.d.              | 36.18 ± 0.62     | 42.7 ± 1.9      | 10.3 ± 0.3      | 27.1 ± 1.2      | -               | -               | [29] |
| FW               | Fed-batch      | 0  | n.d.                | 26.61             | 65               | 6               | 29              | -               | -               | -               |      |
| FW and WAS       | Batch         | 6  | 15                  | 38.27             | 4.603            | 38.9            | 34.7            | 31.5            | 0               | -               | [36] |
| FW and WAS       | (Stirred reactors, Vw = 0.8 L) | 0  | n.d.                | 44.76             | 5.384            | 49              | 15              | 32              | 4               | -               | [37] |
| FW and WAS       | Batch (GSBC, Vw = 2L) | 12.3 ± 1.5 | 24 | 11.9 (in COD) | 7.6 | 5.6 | 7.6 | 12 | 0 | 67.2 |
| FW, Biodiesel WW | Batch (BSR, Vw = 2.5 L) | 12.2 ± 2.2 | 22 | 11.0 (in COD) | 16.2 | 9.3 | 0 | 17.3 | 0 | 57.2 | This study |
| FW, Biodeisel WW | Batch (BSR, Vw = 2.5 L) | 18.4 ± 1.5 | 46 | 22.8 (in COD) | 27.4 | 35.3 | 24.4 | 6.7 | 6.2 | 0 |
| FW, Biodeisel WW | Batch (PBSR, Vw = 1.2 L) | 17.8 ± 1.5 | 41 | 20.3 (in COD) | 28.2 | 38.1 | 23.2 | 6.2 | 4.2 | 0 |
5. Conclusions

In conclusion, an initial pH of 7 allowed for higher acidification yields (41–46%) when compared to a pH of 5 (22–24%). Moreover, the BSR was the reactor more suitable among tested configurations to be used in the acidogenesis of wastes with high solid and salinity content. The fermented broth obtained under those conditions has a greater diversity of VFA with longer chains, and therefore presents a great potential to be used to produce PHA with desirable longer and diverse chains. From an industrial perspective, the implementation of processes with simpler reactors’ configurations, such as the BSR, is advantageous, since it may represent lower investment, operation, and maintenance costs. Nevertheless, this process would be even more apppellative for industrial applications if VFA yields could be improved. Alternative strategies, such as the implementation of micro-aeration [46–48] or the use of conductive materials [49,50], have been the focus of recent studies, where VFA production has been improved.

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