Article

Microwave-Hydrogen Peroxide Assisted Anaerobic Treatment as an Effective Method for Short-Chain Fatty Acids Production from Tannery Sludge

Giulia Adele Tuci [ID], Francesco Valentino [ID], Edoardo Bonato, Paolo Pavan and Marco Gottardo *[ID]

Department of Environmental Science, Informatics and Statistics, Ca' Foscari University of Venice, Mestre, 30172 Venice, Italy
* Correspondence: marco.gottardo@unive.it; Tel.: +39-041-2346654

Abstract: Tannery sludge is disposed of in landfills as it is considered a special residue by the Italian legislation, creating pollution and waste. This paper aims at evaluating the performance of the anaerobic fermentation process to obtain short-chain fatty acids (SCFAs) from this waste. The assessment of the most appropriate conditions, in terms of pH, temperature, initial total solids (TSs) content, and application of oxidizing-thermal pretreatment has been developed. The batch test trials revealed that the combined microwave and hydrogen peroxide (MW-H_2O_2) pretreatment followed by thermophilic conditions gave the best results, in terms of the acidification yield (0.31 $gCOD_{SCFA}/gVS_0$) and maximal SCFA concentration (above 26 g COD_{SCFA}/L). In the tests conducted without pretreatment, the mesophilic temperature should be preferred since the acidification performances were comparable to or even better than their thermophilic counterparts. The SCFA composition analysis showed that in mesophilic fermentation, tannery sludge can generate up to 50% acetic acid (COD_{Ac}/COD_{SCFA}), if previously pretreated (MW-H_2O_2). This research acts as a forerunner for the appropriate handling of this resource, to employ it for the development of a new tannery industry focused on a circular approach, rather than to simply dispose of it in landfills.

Keywords: tannery sludge; anaerobic fermentation; short chain fatty acids (SCFAs); biorefinery

1. Introduction

The concepts of a circular economy and sustainable growth are the main focuses of the European Union's 2030 strategy to meet the Paris agreement requirements [1]. The plan is especially concerned with the improvement of the life cycle of products and the reduction of waste across all sectors. Consequently, resource recovery from wasted materials has gained great importance, especially if such materials are renewable and characterized by a high organic content. In this context of waste production, the tannery sector merits remarkable consideration, as Italy is one of the leading countries in this industry, with a value of production of EUR 3.5 billion in 2020 [2]. In 2020 the production of finished leather products in Italy, was around 97 million square meters; also, according to the Italian tannery industry sustainability report of 2020, Italian tanneries generate an average of 1.65 kg of waste per square meter of leather produced, with 20.8% of it being sludge.

The tanning process is made of different phases both mechanical and chemical and involves the use of chromium salts as tanning agents [3,4], resulting in wastewaters characterized by high amounts of this compound, as well as organic and inorganic substances [5–8] The wastewater produced is commonly treated by centralized industrial wastewater treatment plants, where the physical–chemical treatments and biological treatments produce the so-called tannery sludge as a waste product. The management of this sludge is one of the main issues of the tannery industry, as it is a waste with a high environmental impact, due to the high solids content and the presence of chromium and therefore it must be properly disposed of [9,10]. This sludge is classified as a special non-hazardous residue, according

to Italian legislation [11] and its current destination is to a second-class type B controlled landfill (D.Lgs. 04/06). Therefore, at present times, all the sludge from the tannery industry is disposed of in landfills [4], wasting all the high-quality organic material that characterizes this waste [12] while also having a high economic impact for the industries and significant environmental impacts. Hence, its potential for the exploitation of new valorization routes is still almost totally unexplored.

Previous studies provided some examples of alternative uses of this sludge as a ceramic pigment [3], building material [5], biochar [13], and more, but the only large-scale management method remains landfilling. In terms of biological waste valorization, the anaerobic digestion process is established at full-scale for the treatment of different kinds of organic waste [14]. More recently, within the options of anaerobic waste treatment, many laboratory and pilot scale studies have shown the high potential for the valuable resource recovery, such as the short-chain fatty acids (SCFAs), through the acidogenic fermentation process [15]. SCFAs are products of great commercial interest, as they can be further utilized as building blocks for the chemical industry or as precursors of reduced chemicals and derivatives [14]. Several factors affect the productivity of SCFAs from the acidogenic fermentation of organic waste, namely the pH, temperature and total solids (TSs). Such factors have extensively been analyzed in previous studies on different substrates with sometimes contrasting results, that strongly depend on the specific substrate's characteristics. For what concerns the pH, as explained by [16], the range of pH values to produce SCFAs is between 5.0 and 11.0, but the optimal values depend on the type of waste: for municipal sludge, an alkaline pH is usually preferred, whereas for wastewaters, neutral and acidic conditions tend to favor the production of SCFAs. Likewise, the optimal temperature usually varies, according to the substrate; in [17], a mixture of the organic fraction of the municipal solid waste (OFMSW) and waste activated sludge was utilized to produce SCFAs and biogas under two temperature conditions. Similar results were obtained in the mesophilic (37 °C, 20 g COD_{SCFA}/L) and thermophilic conditions (55 °C, 16.5–31.6 g COD_{SCFA}/L). Differently, working on the OFMSW only, the best results were obtained in the mesophilic conditions (37 °C, 20–24 g COD_{SCFA}/L) [18]. The TS concentration is another parameter that can strongly affect the anaerobic process efficiency. A higher TS level allows for the use of a smaller reactor volume (reducing the investment costs); however, it can reduce the mass transfer, the settling properties and it may affect other rheological properties of the medium (such as the viscosity) [19], decreasing the microbial communities' activities and metabolic pathways, overall.

With a specific reference to tannery sludge, the application of the anaerobic fermentation to extract the SCFAs has not been thoroughly explored yet. To the best of the authors' knowledge, there is only one literature study where tannery sludge utilization is related to SCFA production [10] in this research, the authors produced biochar from tannery sludge and bamboo, which was then added to the tannery sludge to improve the production of SCFAs during the anaerobic fermentation.

As underlined by Zhai and colleagues, the tannery sludge is rich in proteins and macromolecular organic matters, so a mild oxidative treatment was considered a viable option to support the hydrolysis of the organic compound and improve the SCFA production performance. Additionally, partial oxidation is desirable as it reduces the concentration of the S species [20], which are abundant in tannery sludge. Hydrogen peroxide (H_2O_2) is an oxidizing agent that is similar to oxygen in effect but is significantly stronger. Hydrogen peroxide can oxidize organic compounds directly or indirectly through OH free radicals produced by the breakdown of oxygen–oxygen single bond [21]. From a literature review, it was found that the use of H_2O_2 combined with thermal treatment has a higher potential and can be preferred to the simple oxidation, as both treatments can disrupt the sludge floc structure and cause microbial cell rupture, hence solubilizing the particulate organics [22,23]. Moreover, hybrid pretreatments make use of the advantages of the synergism between individual techniques, while also overcoming the drawbacks of individual treatments [23].

Microwave (MW) irradiation was chosen for the thermal pretreatment, as it allows for rapid uniform heating and energy efficiency, while still providing sludge solubilization and the enhancement of the sludge anaerobic digestion, compared to the more costly traditional heating alternatives [23–25].

The purpose of this work is to provide a viable alternative to the wasteful disposal of tannery sludge in landfills and use it instead to produce SCFAs, in line with the EU strategy. A comprehensive acidogenic fermentation batch tests assay has been developed and discussed in this study, where the effect of the temperature, pH, total solids (TSs) concentration, and the combined MW-H_2O_2 pretreatment has been investigated on SCFA production (yield and concentration) and distribution.

2. Materials and Methods

2.1. Substrate Collection and Characteristics

The sludge used for the experiments was a mixture of primary and secondary sludge, obtained from the wastewater treatment plant (WWTP) of Montebello Vicentino (northeast Italy). This WWTP treats about 10,000 m^3/d of industrial wastewater produced by 23 tannery plants. Tannery wastewaters are subjected to physicochemical primary treatment and sedimentation (accomplished inside both the tanneries and the WWTP), and a secondary biological treatment (anoxic/aerobic process) used for the biochemical oxygen demand (BOD) and nitrogen removal from the primary clarified effluent. This sludge was made from approximately 60% *v/v* of sludge derived from the primary treatment and 40% *v/v* from the secondary biological treatment. Furthermore, considering the richness in the protein and macromolecular organic compounds of tannery wastewaters, as well as the salinity level (usually higher than municipal wastewaters), the tannery WWTP accomplishes the nitrogen and carbon removal with a sludge retention time (SRT) above 30 days, substantially higher than the typical SRT of municipal WWTPs.

Regarding the chemical and physical parameters, the tannery sludge showed an average dry matter content of 830 $\pm$ 14 g TSs/kg (total solids) and 590 $\pm$ 4 g VSs/kg (volatile solids). The chemical oxygen demand (COD) was 793 $\pm$ 18 g COD/kg TS; the total phosphorus (TP) and nitrogen (as TKN) were 7.9 $\pm$ 0.4 g P/kg TS and 32.8 $\pm$ 0.9 g N/kg TS, respectively.

2.2. Batch Tests' Rationale

Batch tests were performed, aiming to investigate the effects of the different combinations of the initial pH, temperature, combined MW-H_2O_2 treatment, and total solids (TSs) concentration on SCFA production and distribution from the tannery sludge fermentation. The acidogenic fermentation experiments were conducted under both mesophilic (M, 40 $\pm$ 1 °C) and thermophilic (T, 55 $\pm$ 1 °C) environments, starting from a different TS concentration: namely 80 g TSs/L or 8.0% *w/w* (M8 and T8) and 120 g TSs/L or 12% *w/w* (M12 and T12). Furthermore, a separate series of tests was performed at 80 g TSs/L, with MW-H_2O_2 pretreated sludge (M8-P and T8-P) (Table 1).

Table 1. Summary of the operating conditions investigated in the batch tests and the assigned names.

Operating Conditions	Acidogenic Fermentation Batch Tests (Series)					
	M8	**M12**	**M8-P**	**T8**	**T12**	**T8-P**
Temperature (°C)	40	40	40	55	55	55
Solids' content (gTSs/L)	80	120	80	80	120	80
Range of the initial pH	5–11	5–11	5–11	5–11	5–11	5–11
M_W-H_2O_2 pretreatment [1]	no	no	yes	no	no	yes

[1] H_2O_2 at 35% *w/w*; 80–90 °C, 600 W, 10 min, 0.2 g H_2O_2/g TS.

Each test was prepared by diluting the dried sludge with tap water to reach the required TS concentrations, which have been chosen, based on the performances of a dynamic sludge thickening. For each of the conditions, four different initial pH values (5.0, 7.0, 9.0, and 11.0) were tested. Sodium hydroxide (NaOH) and sulfuric acid (H_2SO_4) were added to reach the initial pH values of 9–11 and 5–7, respectively. The MW-H_2O_2 pretreatment was performed, based on the strategy proposed by [23,24,26], with some adaptations. Namely, after the sludge dilution and the pH adjustment, the bottles were heated to 80 °C in a microwave oven set at 600 W for 10 min; the treatment was carried out with intermittent breaks every 1.30 min to allow for the manual mixing of the sludge [23] and to avoid water loss by evaporation, which was also reduced by keeping the temperature below the boiling point. Once the sludge was allowed to cool to room temperature, the H_2O_2 was added at the chosen dosage of 0.2 g H_2O_2/g TSs, using H_2O_2 at 35%. The bottles were left for 40 min to rest, allowing for the H_2O_2 to react and then were heated again in the MW at 90 °C, to avoid reaching the boiling temperature, with the same method described before.

2.3. Batch Tests' Preparation and Monitoring

Each test was performed in duplicate, in 250 mL glass bottles (working volume of 200 mL) sealed with a cap with a silicon plug. As the anaerobic inoculum, 50 mL of an anaerobic digestate (35 g VS/L) from a full-scale digester, located in Treviso (northeast Italy) and maintained at 37 °C and 55 °C, was used, respectively, for the mesophilic and the thermophilic tests. The resulting substrate/inoculum (S/I) ratio were 8 and 12 for the 8% and the 12% tests, respectively.

The batch fermentation tests were monitored until the plateau in the SCFA production was reached at around three weeks, during which the sludge was manually mixed several times a day. The liquid samples (5.0 mL) were collected for the SCFAs analysis, pH measurements, N-NH_4^+, P-PO_4^{3-}, and soluble COD. The hexavalent Chromium [Cr(VI)] was also analyzed in the liquid phase at the end of the MW-H_2O_2 pretreatment and at the end of each test to quantify any possible [Cr(VI)] release. None of the bottles were opened as the samplings were performed through a syringe injected into the plug.

Following the preliminary adjustment of the pH to reach the four initial pH values, this parameter was only monitored and not corrected anymore, as the aim of this research is to eventually perform these fermentations in a continuous mode and on a larger scale, and the constant adjustment of the pH could not be sustainable in view of the process scale-up.

2.4. Analytical Methods and Calculations

The analyses were conducted, according to the Standard Methods [27] for the TKN, N-NH_4^+ total phosphorus, P-PO_4^{3-}, VS, TS, [Cr(VI)], COD, and alkalinity. The SCFAs were determined using an Agilent 6890 N gas chromatograph equipped with a flame ionization detector (FID) (T = 250 °C). The samples were analyzed through an Agilent J&W DB-FFAP fused silica capillary column (15 m length, 0.53 mm i.D., 0.5 mm film) using hydrogen as a carrier. The inlet was working in split mode, with a split ratio of 20:1. The instrument was programmed with a ramp temperature from 80 °C to 100 °C (10 °C/min). Prior to the GC analyses, the samples were centrifuged at 4.500 rpm for five minutes and the supernatant was filtered at 0.2 mm, using acetate cellulose syringe filters (Whatman).

To assess the nutrient release, the ammonium and phosphate concentrations in the liquid phase ([N-NH_4^+] and [P-PO_4^{3-}]), at the end of experiments, were considered, and their release was calculated, according to the following equations, where TKN and TP represent the initial content in each test: N-NH_4^+ release (%) = [N-NH_4^+]/TKN; P-PO_4^{3-} release (%) = [P-PO_4^{3-}]/TP. In this assessment, the mean concentrations of the nutrients, between the four different initial pH tests, were considered.

The acidogenic fermentation performances were evaluated through the quantification of the SCFA concentration, the ratio between the SCFAs and the soluble COD (both in a COD basin), and the fermentation yield (Y_F), was calculated by the ratio between the

concentration of the produced SCFAs over time ("t"), in terms of the COD, and the concentration of the initial VS of the feedstock (VS_0) in the batch tests: $Y_F = [COD_{SCFA}]_t / VS_0$.

3. Results

3.1. Effect of the Initial pH on the Tannery Sludge Fermentation

Regardless of the initial value, the pH of each test converged to around 7.0 after approximately 4 days, and then settled. For this reason, no significant influence of the pH on the acidification performances was observed. This was due to the observed capacity of the tannery sludge to act as a buffer, resulting in the maintenance of the pH to around 7, even with relatively high concentrations of SCFAs. In fact, the characteristic of this sludge is the presence of lime, which acts as a pH buffer, due to the release of OH^- ions from the residual $Ca(OH)_2$ present in the sludge. Lime is abundantly used in the tannery process during the "Liming" operation, which consists in the utilization of lime ($Ca(OH)_2$) and Na_2S to remove hairs and flesh from the skins and split up the fiber bundles in the raw hides. Moreover, lime can be used to correct the pH of the wastewater before the primary sedimentation treatment. The quantification of the alkalinity confirmed the good buffering capacity of the fermented streams (Tables S1 and S2); based on the whole conducted experimental plan, the alkalinity in the final effluent was in the range of 2.46–2.89 g $CaCO_3$/L, higher than the values reported in the literature for other substrates and related to the buffered fermented streams where the pH was controlled by the digestate recirculation [14].

This characteristic is very noteworthy for future large-scale applications, allowing the process to self-regulate and continue without external intervention to correct the pH. On the contrary, other substrates, such as food waste, are very sensitive to high SCFA concentration as it can cause the pH values to decrease, resulting in toxic conditions for the fermentative bacteria in the reactor [28,29]. In this paper, the results from all the different pH are reported. It is more appropriate to refer to the initial pH, which has been set from 5.0 to 11.0. However, the presence of lime drove the pH around neutrality in less than 5 days (around one-sixth of the total length of the test). Considering this behavior, it a net influence of this parameter on the results has not been observed.

3.2. Production of SCFAs over Time

As shown in the graphs (Figure 1a–c), all the tests in the mesophilic conditions show a behavior of a fast initial SCFAs increase until a plateau in the concentration was reached. The plateau was reached after approximately 10 days, for the series M8, and after approximately 15 days, for the series M12, proving that, from a qualitative point of view, the initial TSs affected the acidification process. The applied pretreatment (series M8-P) prolonged the time for the plateau achievement (15 days, approximately) compared to tests conducted under the same initial TS level (M8). The required time for the maximum SCFA concentration is a measurement of the total exploitation potential of this sludge in terms of the organic matter acidification and future development in the continuous process, by choosing the most appropriate HRT.
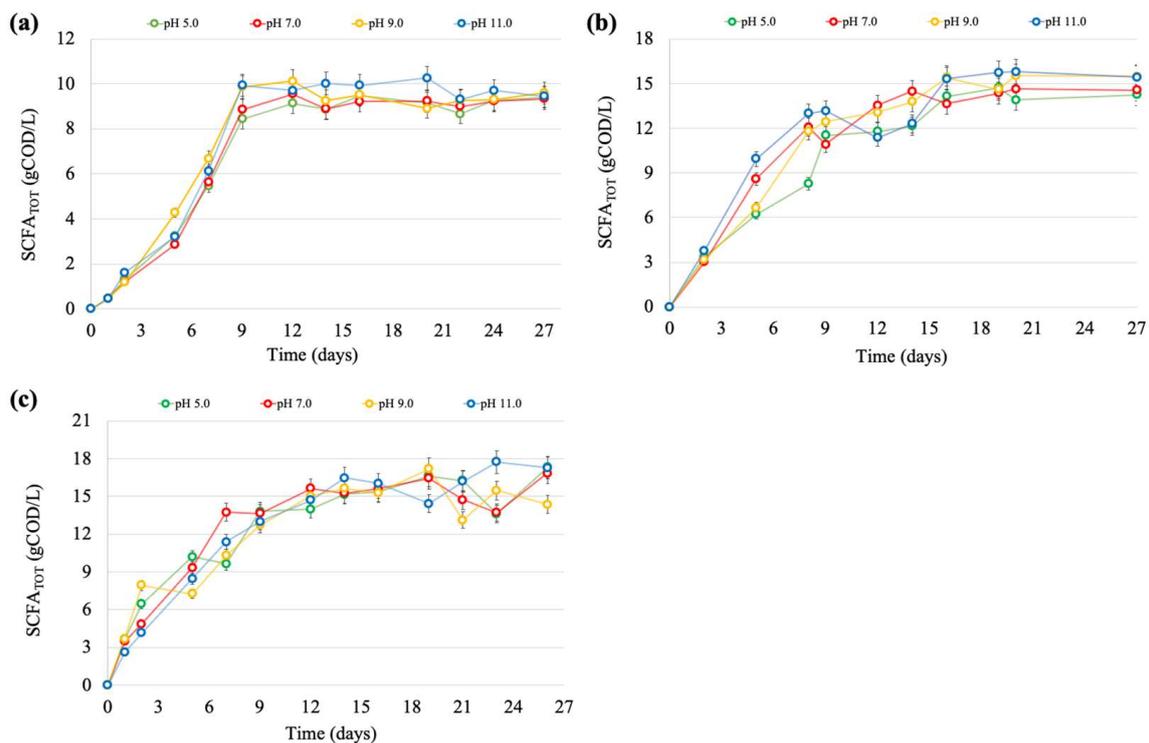
Figure 1. SCFA concentration trends (g COD/L) in the mesophilic batch test M8 (**a**); mesophilic batch test M12 (**b**); mesophilic batch test M8-P (**c**). All three series under the initial pH of 5.0, 7.0, 9.0, and 11.0.

As for the maximum concentration of SCFAs achieved, the series conducted with the pretreated sludge (M8-P) showed the highest values, ranging between 16.8 and 17.7 g COD_{SCFA}/L, demonstrating how the partial oxidation of the organic matter coupled with the microwave treatment, can substantially boost the acidification performances. In fact, H_2O_2 is a strong oxidizing agent (E_0 = 1.78 V) and therefore it can oxidize some organic compounds directly. For instance, H_2O_2 attacks the double bond of an alkene, producing a hydroperoxide; then, the alcohols and ketones are produced from the hydroperoxide. In addition, H_2O_2 can also produce OH free radicals by the breakage of the oxygen–oxygen single bond. The free radicals are extremely reactive; they can attack organic molecules and, therefore, produce another free radical by the chain reaction mechanisms. In the H_2O_2 pretreatment, the tannery sludge was also subjected to the MW to support the mechanism cited above. Hence, such a pretreatment was aimed to reduce the molecular complexity (for example, by breaking the double bonds) of organic compounds and thus support the following biological process.

In addition, the maximum SCFA concentration depended on the initial TS level, being in the range of 9.5–10.25 g COD_{SCFA}/L and 14.6–15.8 g COD_{SCFA}/L in the series M8 and M12, respectively. In practice, the applied pretreatment almost doubled the SCFA production (M8 vs. M8-P); moreover, the inhibition phenomenon can be excluded since the increase of the TS level in M12 led to an increase in the SCFA concentration, compared to the M8 series. In fact, the tannery sludge is generally rich in protein and macromolecular organic matter, whose decomposition leads to the intense ammonium inhibition for the anaerobic bacteria and a low hydrolysis rate of the organic matter [10].

As regards the thermophilic series, the behavior of T8 and T12 was analogous to the mesophilic conditions, even though the SCFAs plateau was reached earlier, in approximately 8 and 12 days for the T8 and T12, respectively, due to the faster kinetic favored by a higher temperature (Figure 2a,b); [30]. Furthermore, in this case, the initial TS level affected the required time to fully exploit the acidification process, in terms of the SCFAs production. By using the pretreated sludge (T8-P series), the SCFAs trend was completely

different from the other thermophilic and mesophilic series (Figure 2c). The T8-P series was characterized by a continuous increase in the production of SCFAs for the whole duration of the experiment, with only a slight slowdown during the final days of the experiment. This can be caused by a synergetic effect of a higher operating temperature (55 °C) and the pre-oxidization of the sludge, as the solubilization of the organic matter is favored by both, triggering an almost continuous fermentation mechanism. From the point of view of this waste valorization under a continuous process operation, this condition could be tricky for the choice of the right HRT, since a value of more than 20 days may strongly affect the economic feasibility of the process.
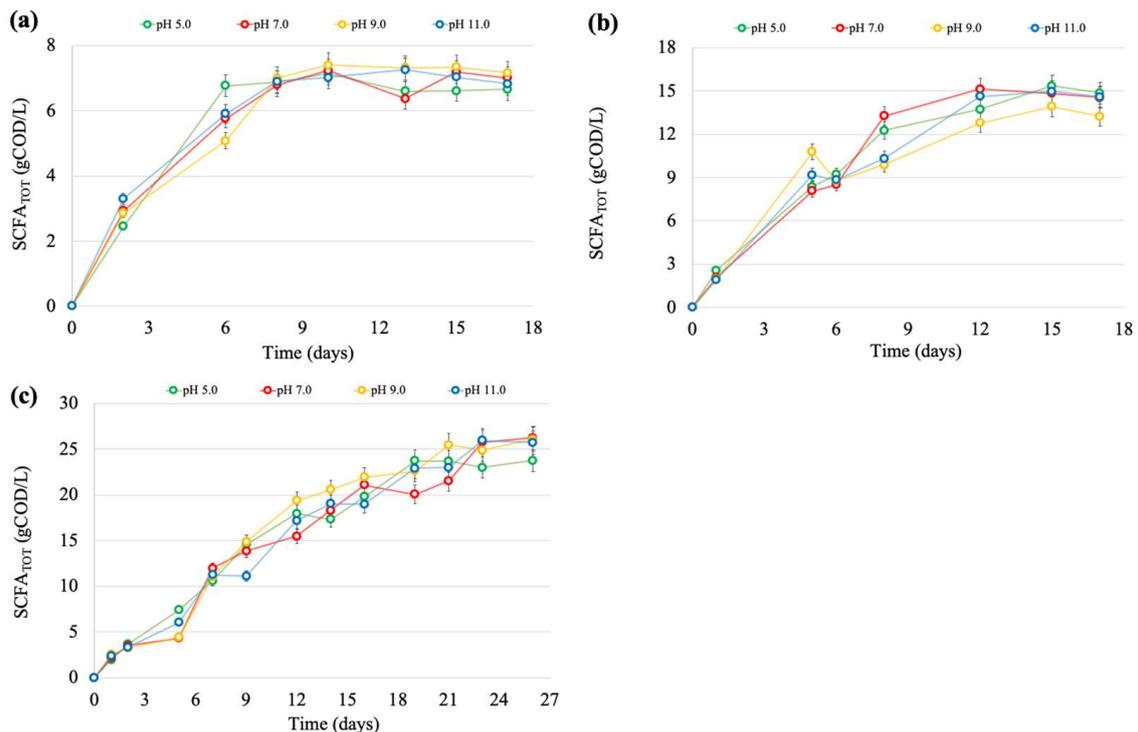

Figure 2. SCFA concentration trends (g COD/L) in the thermophilic batch test T8 (**a**); thermophilic batch test T12 (**b**); thermophilic batch test T8-P (**c**). All three series under the initial pH of 5.0, 7.0, 9.0, and 11.0.

The thermophilic series T8 and T12 gave a lower peak of the SCFA concentration compared to the corresponding mesophilic series M8 and M12. The range of the maximum SCFAs level was 7.1–7.4 and 13.9–15.3 g COD_{SCFA}/L for the series T8 and T12, respectively. Furthermore, in this case, the inhibition phenomenon on the acidogenic culture could be excluded.

Moreover, the thermophilic temperature did not give any benefit, in terms of the SCFAs extraction. Given the different trend observed in the T8-P series, compared to T8 and T12, and the highest SCFA concentrations obtained (23.7–26.2 g COD_{SCFA}/L), the synergistic effect of the thermophilic temperature and the MW-H_2O_2 pretreatment, substantially improved the organic matter acidification, even though the required time for such a performance could be difficult to transfer for the design of a continuous fermentation process.

Apart from the possible improvement for the T8-P condition, mainly addressed to reach the SCFAs plateau in a shorter time, the mesophilic conditions offered a good compromise for the tannery sludge acidification process, with the maximum SCFA concentration between 16.8 and 17.7 g COD_{SCFA}/L, achieved, approximately, in two weeks of the anaerobic condition.

Since the literature is scarcely furnished with data related to SCFAs from tannery sludge, a reasonable comparison can be assessed with municipal sludge-related experiments. Different authors highlighted that SCFA concentration can be higher under mesophilic conditions and with a longer retention time, with an optimal pH for the acidogenic fermentation between 7.0 and 8.0 [31,32]; under the thermophilic condition, the sludge acidogenesis is usually less performing, showing that the available COD_{SOL} remained not converted into SCFAs. Hydrolysis has also been identified as an important step, applied as the hyperthermophilic treatment (70 °C, 24–48 h) for the solubilization of the organics and the increase of their biodegradability [31,32]; in addition, increasing the initial pH had an overall positive effect on the hydrolysis [29].

In the case of the tannery sludge described here, the mesophilic process was better performing. In addition, even if conducted with the combined MW-H_2O_2 treatment, the hydrolysis of the tannery sludge also improved the acidification process under both the mesophilic and thermophilic conditions. No considerations can be made about the pH effect, since the presence of lime, a particular characteristic of this sludge, acted as a buffer and maintained the pH around 7.0 for the whole duration of the experiments.

The maximal SCFA concentration obtained is higher than the values reported in the literature for the municipal sludge (primary, secondary, or mixture) [31–34].

3.3. SCFAs/COD_{SOL} Ratio

The conversion of the waste organic matter into SCFAs generally leads to an increase in the SCFAs/COD_{SOL} ratio, compared to the untreated feedstock [34]. However, the degradation of the organics could also cause a progressive increase in their solubilization and, in turn, in the COD_{SOL} level, not always sustained by a parallel acidification process. This fact is generally linked to the application of the thermophilic condition for a certain type of substrate [14]. Hence, in the acidogenic fermentation process, it is important to have a good balance between the organic matter solubilization and acidification processes. The parameter SCFAs/COD_{SOL} ratio is an important indication of the technical feasibility of the waste fermentation process. To the best of the authors' knowledge, the literature does not furnish data on this parameter related to tannery sludge utilization; hence, this work has started to cover such a gap in the knowledge. Figure 3a shows the SCFAs/COD_{SOL} ratio in the mesophilic series, calculated at the maximum SCFA concentration achieved (in most cases, at the end of the experiment). All three conditions showed similar results, with a range of variability of 0.70–74 COD/COD in M8, 0.72–0.75 COD/COD in M12, and 0.70–0.76 COD/COD in M8-P (average data). Given the standard deviation within such variability ranges, apparently, the initial solids content and the application of the combined pretreatment did not have a substantial impact on the SCFAs/COD_{SOL} ratio.
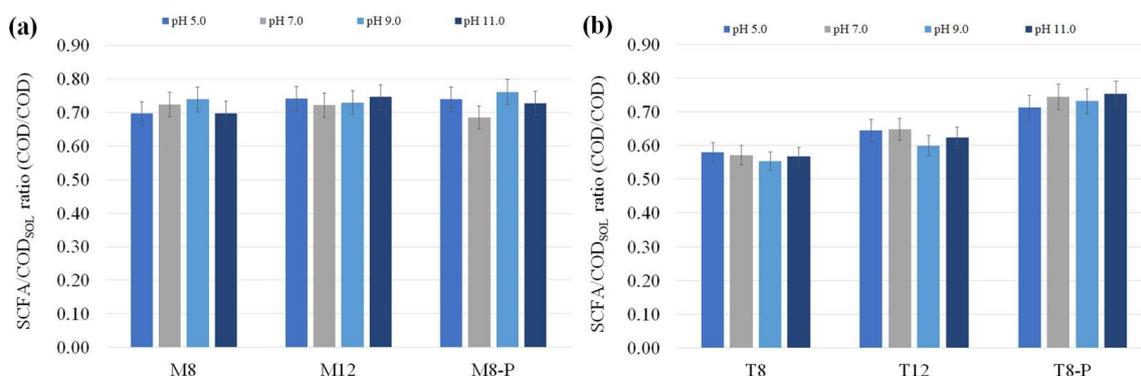

Figure 3. SCFAs/COD_{SOL} ratio (COD/COD) of the mesophilic batch series M8, M12, and M8-P under the initial pH 5.0, 7.0, 9.0, and 11.0 (**a**); SCFAs/COD_{SOL} ratio (COD/COD) of the thermophilic batch series T8, T12, and T8-P under the initial pH 5.0, 7.0, 9.0, and 11.0 (**b**).

Figure 3b shows the results for the same parameter, obtained in the thermophilic tests. The T8 series was the only condition showing values below 0.60 COD/COD, within the narrow range of 0.55–0.58 COD/COD and even lower compared to the corresponding M8 series. The T12 showed a slight increase compared to the T8 series (0.60–0.65 COD/COD), but still lower than the results obtained in M12. Only in the T8-P series did the SCFAs/COD_{SOL} reach above 0.70 COD/COD, and comparable to the values obtained in the mesophilic M8-P series (0.71–0.75 COD/COD). Based on these considerations, it is reasonable to assume that the MW-H_2O_2 pretreatment brought substantial benefits to the thermophilic acidification of the COD_{SOL}, which remained unconverted in a higher percentage under both T8 and T12 series. Overall, the mesophilic condition appeared preferable for the COD_{SOL} conversion into SCFAs, independently from the initial TS level; however, the MW-H_2O_2 pretreatment made the two applied temperature regimes equivalent, in terms of the SCFAs/COD_{SOL} ratio.

Even though these conversion performances can be improved, these results demonstrated that more than 70% of the organic matter contained in the tannery sludge can be easily converted into SCFAs. Other carbon sources, such as the organic fraction of municipal solid waste (OFMSW) and/or different food by-products, are characterized by a high content of putrescible matter; compared to tannery sludge, these carbon sources can be enriched in SCFAs up to 90% of the soluble COD [35]. Moreover, the acidogenic fermentation of the municipal sewage sludge is generally characterized by similar results, compared to this work (0.51–0.69 COD/COD; [33]), showing discontinuous improvements when the fermentation process is conducted on thermally hydrolyzed sludge (0.46–0.75 COD/COD; [32,36]).

3.4. Fermentation Yield (Y_F)

The importance of the Y_F is related to the quantity of SCFAs potentially recovered from a waste carbon source, in terms of the mass balance assessment (not foreseen in this study) in a full-scale scenario. Hence, the quantification of this parameter is crucial for the further economic evaluation of the unborn tannery-based biorefinery value chain. Figure 4a,b shows the Y_F in all of the performed series.
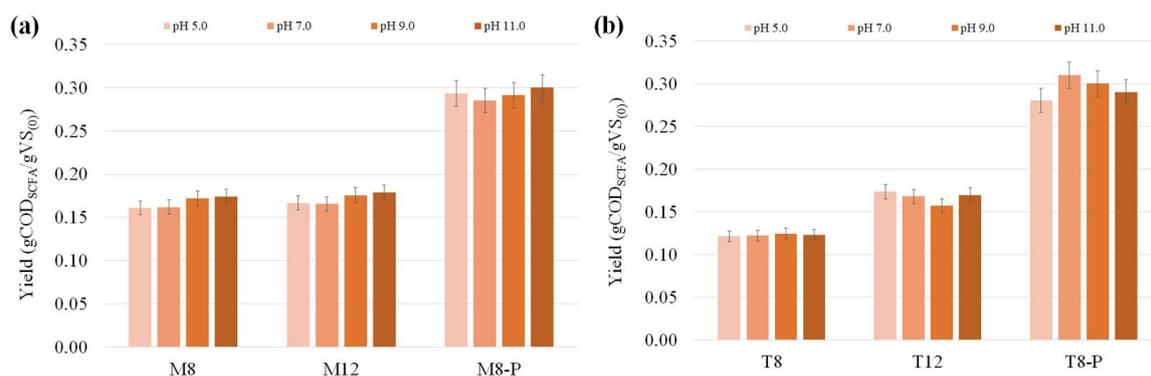

Figure 4. Fermentation yield values (Y_F; g COD_{SCFA}/g VS_0) in the mesophilic batch series M8, M12, and M8-P (**a**); fermentation yield values (Y_F; g COD_{SCFA}/g VS_0) in the thermophilic batch series T8, T12, and T8-P (**b**).

Under the mesophilic conditions, the Y_F was not affected by the increase in the VSs, being in the range 0.16–0.17 g COD_{SCFA}/g VS_0 in the series M8, and 0.16–0.17 g COD_{SCFA}/g VS_0 in the series M12. On the contrary, the thermophilic series T8 and T12 showed different performances: 0.12 g COD_{SCFA}/g VS_0 and 0.16–0.17 g COD_{SCFA}/g VS_0 for T8 and T12 series, respectively. A higher solids concentration and thermophilic environment (T12) probably boosted the solids solubilization and their conversion into SCFAs, compared to the less favorable T8 condition, where the initial VS amount was lower. The Y_F of non-pretreated tannery sludge was found to be comparable to or higher than that of the

municipal sewage sludge (0.06 g COD_{SCFA}/g VS_0) or the primary/sewage sludge mixture (0.11 g COD_{SCFA}/g VS_0) in the mesophilic fermentation [37]. However, other mesophilic investigations on sewage sludge reported higher yields, compared to those reported here for the non-pretreated tannery sludge (0.21–0.33 g COD_{SCFA}/g VS_0; [34]).

The chosen pretreatment greatly improved the Y_F for both the mesophilic and thermophilic series, showing a range of 0.28–0.30 g COD_{SCFA}/g VS_0 and 0.28–0.31 g COD_{SCFA}/g VS_0, respectively in M8-P and T8-P. This showed that the combined pretreatment, as well as the H_2O_2 dosage, was undoubtedly useful to increase the tannery sludge fermentability. Under all of the conditions investigated, these numbers represent the optimal SCFA yields, even higher than the yields obtained in the mesophilic fermentation of the thermally hydrolyzed (TH) municipal sludge (0.22 g COD_{SCFA}/g VS_0), where the authors stated that the advantage of the TH-sludge in the COD solubilization overcame its disadvantage of a low biodegradability [32].

Overall, these values are in line (and even higher) with the yields from other experiments dealing with municipal sewage sludge, usually considered suitable for fermentation or AD systems, revealing that tannery sludge can be employed for this process as well with promising results for further continuous trials and future scale-ups of the technology.

3.5. Nutrients' Release and Quantification

The ammonium and phosphate concentrations in the liquid fraction were measured to estimate the nutrient release and their potential recovery. The release of this macro-nutrient was affected only by the application of the combined MW-H_2O_2 pretreatment; no effects were quantified by the temperature, pH, or the initial TS level.

In summary, the nutrients' release in the mesophilic conditions led to final ammonium and phosphate concentrations of 781 ± 11 (M8), 1181 ± 38 (M12), 1605 ± 44 (M8-P) mg N-NH_4^+/L, and 1.16 ± 0.04 (M8), 2.00 ± 0.03 (M12), 2.40 ± 0.08 (M8-P) mg P-PO_4^{3-}/L (average data calculated by considering the tests at the different initial pH).

Similarly, in the thermophilic tests, the final ammonium and phosphate concentrations were 789 ± 19 (T8), 1216 ± 29 (T12), 1586 ± 40 (T8-P) mg N-NH_4^+/L, and 2.3 ± 0.2 (T8), 2.7 ± 0.1 (T12), 2.8 ± 0.1 (T8-P) mg P-PO_4^{3-}/L.

Considering the N-NH_4^+ release, all the tests conducted with non-pretreated sludge gave values around 30%; in particular, the ammonium released in M8 and M12 were 29.6% and 29.8%, respectively; in the T8 and T12 series, it was equal to 30.0 and 30.9%. These results proved that the maximum release of nitrogen from this substrate was roughly 30%, regardless of the TS content and the temperature applied. M8-P and T8-P proved, once again, that the MW-H_2O_2 pretreatment can improve this process overall, as the NH_4-N release values reached 61.5% for the M8-P and 73.8% for the T8-P.

Definitively lower performances were obtained for the phosphorous release, which showed values remarkably lower than the ammonium release, and always below 1.0%, regardless of the TS level, temperature, or the pretreatment application.

Based on these results, the application of a pretreatment must be considered for a series of benefits which cannot be differently reached. Both the ammonium release (and potential recovery), as well as the SCFA production, have been positively affected by the MW-H_2O_2 pretreatment and future investigations into a continuous reactor, will be also addressed the TS and VS reduction (which in turn affects the tannery sludge disposal cost).

3.6. SCFAs Composition

As for the other parameters, independent from the tested conditions (temperature, VS level, MW-H_2O_2 pretreatment), no difference between the different initial pH, has been observed for the SCFA spectrum. The following Figures 5 and 6 represent the average SCFA profile corresponding to the maximum yield for each series.
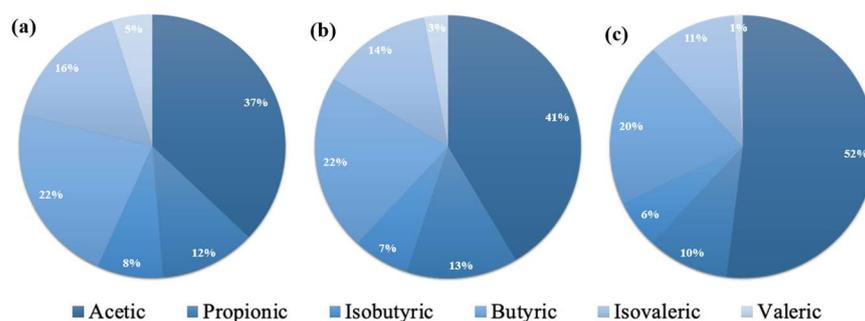
Figure 5. Composition in terms of the COD_{SOL} percentage of the SCFAs obtained from the mesophilic batch series M8 (**a**), M12 (**b**), and M8-P (**c**) as the average of all the initial pH.
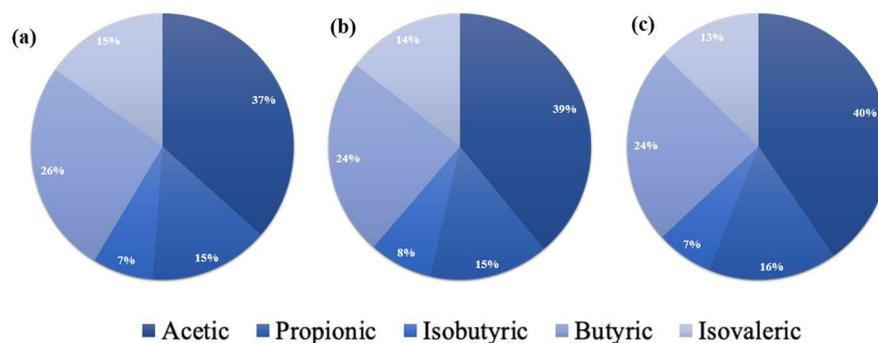

Figure 6. Composition in terms of the COD_{SOL} percentage of the SCFAs obtained from the thermophilic batch series T8 (**a**), T12 (**b**), and T8-P (**c**) as the average of all the initial pH.

The mesophilic series M8 and M12 showed quite similar values (Figure 5), with a dominance of acetic (37–41% COD_{Ac}/COD_{SCFA}, respectively, in the M8 and M12 series) and butyric acid (22% COD_{But}/COD_{SCFA}, in both series), followed by isovaleric (16–14% COD_{Isov}/COD_{SCFA}), propionic (12–13% COD_{Pr}/COD_{SCFA}) and a lower amount of isobutyric (8–7% COD_{Isob}/COD_{SCFA}) and valeric (5–3% COD_{Val}/COD_{SCFA}) acid. The MW-H_2O_2 pretreatment caused a shift in the composition, towards acetic acid, making up 52% of the total, at the expense of the other SCFAs, which all decreased in the M8-P series. Apparently, in addition to the increase in biodegradability, the MW-H_2O_2 pretreatment moved the metabolic mesophilic fermentation pathways more forward to the last fermentation products (e.g., acetic acids).

Moreover, in the thermophilic series, neither the TS nor the pretreatment changed the SCFA composition (Figure 6). The dominating acids for T8, T12, and T8-P, remained acetic (37, 39, and 40% COD_{Ac}/COD_{SCFA}, respectively), and butyric (26, 24 and 24% COD_{But}/COD_{SCFA}), followed by isovaleric (15, 14 and 13% COD_{Isov}/COD_{SCFA}), propionic (15, 15 and 16% COD_{Pr}/COD_{SCFA}), and a lower amount of isobutyric (7, 8 and 7% COD_{Isob}/COD_{SCFA}). Compared to the mesophilic trials, the higher temperature applied in T8, T12, and T8-P, annulled the MW-H_2O_2 impact on SCFA distribution observed in the M8-P series. Noticeably, valeric acid completely disappeared, compared to the corresponding mesophilic series. This was the only substantial change, caused by the two applied temperature regimes.

Compared to the composition of the sewage sludge fermentation liquids, the tannery sludge could generate more acetic acid (up to 50% COD_{Ac}/COD_{SCFA}) under the specific condition of the MW-H_2O_2 pretreatment, followed by the mesophilic acidification process. The SCFA distribution observed in the other tested conditions was quite similar to those distributions described in the literature, from the batch sewage sludge acidification without a pH-control strategy, thermally, and non-thermally pretreated: acetic (30–40% COD_{Ac}/COD_{SCFA}) and butyric (20–30% COD_{But}/COD_{SCFA}) acids are generally dominating, with a lower amount of propionic (15–22% COD_{Pr}/COD_{SCFA}), isobutyric,

valeric, and isovaleric were roughly present between 10 and 15% COD/COD$_{SCFA}$ [32,34,37]. Valeric acid was also reported with a higher percentage under the mesophilic (26–27% COD$_{Val}$/COD$_{SCFA}$) and thermophilic (21–24% COD$_{Val}$/COD$_{SCFA}$) sewage sludge fermentation processes [32].

3.7. Perspective of the Tannery Sludge Utilization in a New Biorefinery Value-Chain

Due to its high chromium ion content (40–80 g/kg), tannery sludge is considered hazardous waste and its management represents a big challenge for the leather industry [38]. However, the recent strategies in the European Union (EU) are focused to set the transition toward a circular economy to maintain the value of products/materials/resources for the longest time possible, together with the objective of waste minimization. The development of a biorefinery represents a method to approach resource valorization, aiming to use available renewable feedstock to provide high-value marketable products while minimizing the energy consumption and waste generation. In this sense, the potential for microbial fermentation and the recovery of SCFAs from tannery sludge has never been investigated in detail, and this study furnishes a deep evaluation. The two tables (Tables S1 and S2) summarize all the results obtained in this work from the mesophilic and thermophilic experiments. Within a future and desirable biorefinery scenario for the tannery waste valorization, apart from the possible improvements of the Y_F, further efforts must be devoted to continuous experiments and the scaled-up approach. The results obtained in this study demonstrated the feasibility to recover a significant amount of SCFAs in the liquid phase, with no risk related to the Cr(VI) release. The Cr(VI) concentration quantified at the end of the MW-H$_2$O$_2$ pretreatment was below the LOQ of 0.03 mg/L; the same results were obtained at the end of each fermentation experiment, demonstrating that no Cr(VI) release occurred for the whole tests' length, under the two investigated temperatures.

Given the high content of the macromolecular organic matters, the consideration of a multi-purpose biorefinery approach is reasonable and it could allow to recover, not only bio-based chemicals (such as SCFAs), but also interesting biofuel, such as biohythane [35]. However, in addition to other biorefinery scenarios developed at a pilot or semi-demonstrative scale on urban and agri-food waste [39,40], the chromium issue in the tannery waste biorefinery, must be considered its distribution among the generated fluxes and fate in the final bio-products, if any. In fact, it is noteworthy that producing biobased molecules instead of biofuels increases the economic added value, contributing to the development of a real and effective bioeconomy scenario [41].

4. Conclusions

This study aimed at evaluating the performance of tannery sludge in an anaerobic fermentation process to obtain SCFAs, and to assess the most appropriate conditions in terms of the pH, operating temperature, VS content, and oxidizing and heating pretreatments. The batch test trials revealed that the combined MW-H$_2$O$_2$ pretreatment associated with the mesophilic or thermophilic conditions gave comparable results, in terms of yield (being 0.28–0.30 and 0.28–0.31 g COD$_{SCFA}$/g VS$_0$ in the mesophilic and thermophilic series, respectively) and the SCFAs/COD$_{SOL}$ ratio (the SCFA amount counted for more than 70% of the soluble COD). The SCFAs production, over time, showed maximum values in the range of 16,800–17,700 mg COD$_{SCFA}$/L (achieved in approximately two weeks) and 23,700–26,200 mg COD$_{SCFA}$/L (approximately achieved in three weeks) respectively for the mesophilic and thermophilic trials. Lower performances were related to the absence of the pretreatment (especially for the Y_F) and no substantial effect was found to be linked to the different initial VS levels.

The performed tests revealed a particular characteristic of tannery sludge, which was its capacity to act as a buffer, resulting in the maintenance of a neutral pH even with high concentrations of SCFAs, meaning that the tannery sludge fermentation does not need the use of chemicals for the pH control and the fermentative bacteria are far from being at risk of becoming inhibited by sudden pH drops.

Overall, this work represents the first and deep evaluation of microbial SCFA production from tannery sludge and future efforts will be dedicated to the investigation of the pretreatment and continuous process configuration, as a part of the mandatory action for the maximization of the resource recovery from this abundant and polluting waste, which is currently wasted completely, with dangerous environmental consequences and high economic expenses for the producers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr10112167/s1, Table S1: Summary of the main results (average data) obtained in the mesophilic (M8, M12, M8-P) anaerobic fermentation of the tannery sludge; Table S2: Summary of the main results (average data) obtained in the thermophilic (T8, T12, T8-P) anaerobic fermentation of the tannery sludge.

References

1. 2030 Climate & Energy Framework. Available online: https://ec.europa.eu/clima/policies/strategies/2030_en (accessed on 26 May 2022).
2. UNIC Italian Tanneries. *Business Overview on Italian Tanning Industry*; UNIC Italian Tanneries: San Miniato, Italy, 2020.
3. Abreu, M.A.; Toffoli, S.M. Characterization of a Chromium-Rich Tannery Waste and Its Potential Use in Ceramics. *Ceram. Int.* **2009**, *35*, 2225–2234. [CrossRef]
4. Alibardi, L.; Cossu, R. Pre-Treatment of Tannery Sludge for Sustainable Landfilling. *Waste Manag.* **2016**, *52*, 202–211. [CrossRef] [PubMed]
5. Basegio, T.; Berutti, F.; Bernardes, A.; Pérez, C.; Bergmann, P. Environmental and Technical Aspects of the Utilisation of Tannery Sludge as a Raw Material for Clay Products. *J. Eur. Ceram. Soc.* **2002**, *22*, 2251–2259. [CrossRef]
6. Liu, H.; Wang, Y.; Zhang, H.; Huang, G.; Yang, Q.; Wang, Y. Synchronous Detoxification and Reduction Treatment of Tannery Sludge Using Cr (VI) Resistant Bacterial Strains. *Sci. Total Environ.* **2019**, *687*, 34–40. [CrossRef]
7. Mpofu, A.B.; Welz, P.J.; Oyekola, O.O. Anaerobic Digestion of Secondary Tannery Sludge: Optimisation of Initial PH and Temperature and Evaluation of Kinetics. *Waste Biomass Valorization* **2020**, *11*, 873–885. [CrossRef]
8. Mpofu, A.B.; Kibangou, V.A.; Kaira, W.M.; Oyekola, O.O.; Welz, P.J. Anaerobic Co-Digestion of Tannery and Slaughterhouse Wastewater for Solids Reduction and Resource Recovery: Effect of Sulfate Concentration and Inoculum to Substrate Ratio. *Energies* **2021**, *14*, 2491. [CrossRef]
9. Silva, J.D.C.; Leal, T.T.B.; Araújo, A.S.F.; Araujo, R.M.; Gomes, R.L.F.; Melo, W.J.; Singh, R.P. Effect of Different Tannery Sludge Compost Amendment Rates on Growth, Biomass Accumulation and Yield Responses of Capsicum Plants. *Waste Manag.* **2010**, *30*, 1976–1980. [CrossRef]
10. Zhai, S.; Li, M.; Xiong, Y.; Wang, D.; Fu, S. Dual Resource Utilization for Tannery Sludge: Effects of Sludge Biochars (BCs) on Volatile Fatty Acids (VFAs) Production from Sludge Anaerobic Digestion. *Bioresour. Technol.* **2020**, *316*. [CrossRef]
11. Ronchi Decree. 1997. Available online: https://www.parlamento.it/parlam/leggi/deleghe/97022dl.htm (accessed on 24 March 2022).
12. Pietrelli, L.; Ippolito, N.M.; Reverberi, A.P.; Vocciante, M. Heavy Metals Removal and Recovery from Hazardous Leather Sludge. *Chem. Eng. Trans.* **2019**, *76*, 1327–1332. [CrossRef]

13. Skrzypczak, D.; Szopa, D.; Mikula, K.; Izydorczyk, G.; Baśladyńska, S.; Hoppe, V.; Pstrowska, K.; Wzorek, Z.; Kominko, H.; Kułażyński, M.; et al. Tannery Waste-Derived Biochar as a Carrier of Micronutrients Essential to Plants. *Chemosphere* **2022**, *294*, 133720. [CrossRef]

14. Valentino, F.; Munarin, G.; Biasiolo, M.; Cavinato, C.; Bolzonella, D.; Pavan, P. Enhancing Volatile Fatty Acids (VFA) Production from Food Waste in a Two-Phases Pilot-Scale Anaerobic Digestion Process. *J. Environ. Chem. Eng.* **2021**, *9*, 106062. [CrossRef]

15. Naresh Kumar, A.; Sarkar, O.; Chandrasekhar, K.; Raj, T.; Narisetty, V.; Mohan, S.V.; Pandey, A.; Varjani, S.; Kumar, S.; Sharma, P.; et al. Upgrading the Value of Anaerobic Fermentation via Renewable Chemicals Production: A Sustainable Integration for Circular Bioeconomy. *Sci. Total Environ.* **2022**, *806*, 150312. [CrossRef] [PubMed]

16. Lee, W.S.; Chua, A.S.M.; Yeoh, H.K.; Ngoh, G.C. A Review of the Production and Applications of Waste-Derived Volatile Fatty Acids. *Chem. Eng. J.* **2014**, *235*, 83–99. [CrossRef]

17. Valentino, F.; Moretto, G.; Gottardo, M.; Pavan, P.; Bolzonella, D.; Majone, M. Novel Routes for Urban Bio-Waste Management: A Combined Acidic Fermentation and Anaerobic Digestion Process for Platform Chemicals and Biogas Production. *J. Clean. Prod.* **2019**, *220*, 368–375. [CrossRef]

18. Soomro, A.F.; Abbasi, I.A.; Ni, Z.; Ying, L.; Liu, J. Influence of Temperature on Enhancement of Volatile Fatty Acids Fermentation from Organic Fraction of Municipal Solid Waste: Synergism between Food and Paper Components. *Bioresour. Technol.* **2020**, *304*, 122980. [CrossRef]

19. Battista, F.; Almendros, M.G.; Rousset, R.; Boivineau, S.; Bouillon, P.A. Enzymatic Hydrolysis at High Dry Matter Content: The Influence of the Substrates' Physical Properties and of Loading Strategies on Mixing and Energetic Consumption. *Bioresour. Technol.* **2018**, *250*, 191–196. [CrossRef] [PubMed]

20. Horn, E.J.; Oyekola, O.O.; Welz, P.J.; van Hille, R.P. Biological Desulfurization of Tannery Effluent Using Hybrid Linear Flow Channel Reactors. *Water* **2022**, *14*, 32. [CrossRef]

21. Woodard and Curran. Chapter 7 Methods for Treating Wastewater from Industry. In *Industrial Waste Treatment Handbook*; Woodard and Curran: Portland, ME, USA, 2006; ISBN 978-0-7506-7963-3. [CrossRef]

22. Tyagi, V.K.; Lo, S.L. Application of Physico-Chemical Pretreatment Methods to Enhance the Sludge Disintegration and Subsequent Anaerobic Digestion: An up to Date Review. *Rev. Environ. Sci. Biotechnol.* **2011**, *10*, 215–242. [CrossRef]

23. Ambrose, H.W.; Chin, C.T.L.; Hong, E.; Philip, L.; Suraishkumar, G.K.; Sen, T.K.; Khiadani, M. Effect of Hybrid (Microwave-H_2O_2) Feed Sludge Pretreatment on Single and Two-Stage Anaerobic Digestion Efficiency of Real Mixed Sewage Sludge. *Process Saf. Environ. Prot.* **2020**, *136*, 194–202. [CrossRef]

24. Liu, J.; Jia, R.; Wang, Y.; Wei, Y.; Zhang, J.; Wang, R.; Cai, X. Does Residual H_2O_2 Result in Inhibitory Effect on Enhanced Anaerobic Digestion of Sludge Pretreated by Microwave-H_2O_2 Pretreatment Process? *Environ. Sci. Pollut. Res.* **2017**, *24*, 9016–9025. [CrossRef]

25. Özön, E.; Erdinçler, A. Effects of Microwave, H_2O_2/MW and H_2O_2/Heat Pre-Treatments on the Methane Production from Wastewater Sludges: Experimental and Modeling Approach. *Environ. Sci. Pollut. Res.* **2019**, *26*, 35411–35421. [CrossRef] [PubMed]

26. Liu, J.; Yang, M.; Zhang, J.; Zheng, J.; Xu, H.; Wang, Y.; Wei, Y. A Comprehensive Insight into the Effects of Microwave-H_2O_2 Pretreatment on Concentrated Sewage Sludge Anaerobic Digestion Based on Semi-Continuous Operation. *Bioresour. Technol.* **2018**, *256*, 118–127. [CrossRef] [PubMed]

27. Apha Awwa, W.E.F. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association, American Water Works Association, and Water Environment Federation: Washington, DC, USA, 2005.

28. Franke-Whittle, I.H.; Walter, A.; Ebner, C.; Insam, H. Investigation into the Effect of High Concentrations of Volatile Fatty Acids in Anaerobic Digestion on Methanogenic Communities. *Waste Manag.* **2014**, *34*, 2080–2089. [CrossRef] [PubMed]

29. Zhang, L.; Tsui, T.H.; Loh, K.C.; Dai, Y.; Tong, Y.W. Acidogenic Fermentation of Organic Wastes for Production of Volatile Fatty Acids. In *Biomass, Biofuels, Biochemicals: Microbial Fermentation of Biowastes*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 343–366. [CrossRef]

30. Vidal-Antich, C.; Perez-Esteban, N.; Astals, S.; Peces, M.; Mata-Alvarez, J.; Dosta, J. Assessing the Potential of Waste Activated Sludge and Food Waste Co-Fermentation for Carboxylic Acids Production. *Sci. Total Environ.* **2021**, *757*, 143763. [CrossRef] [PubMed]

31. Niero, L.; Morgan-Sagastume, F.; Lagerkvist, A. Accelerating Acidogenic Fermentation of Sewage Sludge with Ash Addition. *J. Environ. Chem. Eng.* **2021**, *9*, 106564. [CrossRef]

32. Zhang, D.; Jiang, H.; Chang, J.; Sun, J.; Tu, W.; Wang, H. Effect of Thermal Hydrolysis Pretreatment on Volatile Fatty Acids Production in Sludge Acidification and Subsequent Polyhydroxyalkanoates Production. *Bioresour. Technol.* **2019**, *279*, 92–100. [CrossRef]

33. Presti, D.; Cosenza, A.; Capri, F.C.; Gallo, G.; Alduina, R.; Mannina, G. Influence of Volatile Solids and PH for the Production of Volatile Fatty Acids: Batch Fermentation Tests Using Sewage Sludge. *Bioresour. Technol.* **2021**, *342*, 125853. [CrossRef]

34. Morgan-Sagastume, F.; Hjort, M.; Cirne, D.; Gérardin, F.; Lacroix, S.; Gaval, G.; Karabegovic, L.; Alexandersson, T.; Johansson, P.; Karlsson, A.; et al. Integrated Production of Polyhydroxyalkanoates (PHAs) with Municipal Wastewater and Sludge Treatment at Pilot Scale. *Bioresour. Technol.* **2015**, *181*, 78–89. [CrossRef]

35. Gottardo, M.; Bolzonella, D.; Adele Tuci, G.; Valentino, F.; Majone, M.; Pavan, P.; Battista, F. Producing Volatile Fatty Acids and Polyhydroxyalkanoates from Foods By-Products and Waste: A Review. *Bioresour. Technol.* **2022**, *361*, 127716. [CrossRef]

36. Liu, H.; Han, P.; Liu, H.; Zhou, G.; Fu, B.; Zheng, Z. Full-Scale Production of VFAs from Sewage Sludge by Anaerobic Alkaline Fermentation to Improve Biological Nutrients Removal in Domestic Wastewater. *Bioresour. Technol.* **2018**, *260*, 105–114. [CrossRef]
37. Morgan-Sagastume, F.; Valentino, F.; Hjort, M.; Cirne, D.; Karabegovic, L.; Gerardin, F.; Johansson, P.; Karlsson, A.; Magnusson, P.; Alexandersson, T.; et al. Polyhydroxyalkanoate (PHA) Production from Sludge and Municipal Wastewater Treatment. *Water Sci. Technol.* **2014**, *69*, 177–184. [CrossRef] [PubMed]
38. Jin, M.; Lian, F.; Xia, R.; Wang, Z. Formulation and Durability of a Geopolymer Based on Metakaolin/Tannery Sludge. *Waste Manag.* **2018**, *79*, 717–728. [CrossRef] [PubMed]
39. Righetti, E.; Nortilli, S.; Fatone, F.; Frison, N.; Bolzonella, D. A Multiproduct Biorefinery Approach for the Production of Hydrogen, Methane and Volatile Fatty Acids from Agricultural Waste. *Waste Biomass Valorization* **2020**, *11*, 5239–5246. [CrossRef]
40. Moretto, G.; Lorini, L.; Pavan, P.; Crognale, S.; Tonanzi, B.; Rossetti, S.; Majone, M.; Valentino, F. Biopolymers from Urban Organic Waste: Influence of the Solid Retention Time to Cycle Length Ratio in the Enrichment of a Mixed Microbial Culture (MMC). *ACS Sustain. Chem. Eng.* **2020**, *8*, 14531–14539. [CrossRef]
41. Uçkun Kiran, E.; Trzcinski, A.P.; Liu, Y. Platform Chemical Production from Food Wastes Using a Biorefinery Concept. *J. Chem. Technol. Biotechnol.* **2015**, *90*, 1364–1379. [CrossRef]