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Sustainable Development
and the Environment**

Final Thesis

Anaerobic Processes for Tannery Sludge Valorization

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1. Introduction

The Paris agreement (COP 21, December 2015) highlighted climate change and the countries ability to deal with its impacts as its main focus; with the Green Deal, Europe aims to overcome these challenges, becoming a modern, resource-efficient, and competitive economy (ec.europa.eu). In this context, the principles of Sustainable development and Circular Economy, such as the recovery of resources from waste, are crucial. Wastes with high organic loads, in particular, can be treated as a renewable feedstock, as they are continuously produced as byproducts of several urban and industrial activities, such as the tanning industry. The tannery sector is considered one of the most polluting industrial activities in the world (Mannucci et al. 2010), and Italian production is among the most remarkable (16% of global turnover), being the first in Europe (66% of European turnover) (Alibardi et al. 2016). Industries are located mainly in four regions: Veneto, Tuscany, Campania, and Lombardy. 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 total production in 2020 was around 97 million square meters, so we can estimate a total of 33,3 thousand tons of sludge produced. Based on the raw material, process, and chemicals used, which all vary according to the time of the year and the area, this waste may contain a complex mixture of both organic and inorganic pollutants (Mwinyihija et al. 2010) that can cause serious environmental impacts on water, land and atmosphere (Giaccherini et al. 2017). Enforcing the application of Circular Economy principles in this sector will lead to a significant reduction of the volume of sludge to be disposed of, as well as an increase in the potential of recovery of added value products such as short chain fatty acids (SCFAs).

SCFAs nowadays are mainly produced by chemical synthesis of raw petroleum, which is a non-renewable source (Volker et al. 2014), and microbial cultures feed with pure sugars (Feng et al. 2010, Zhu and Yang 2004), which can compete with the food market. These practices inevitably lead to the emission of greenhouse gasses (GHG); as an example, the production of 1 ton of acetic acid via petrochemical synthesis emit 3.3 tons of CO_2-eq cradle-to-grave (Atasoy et al. 2018). The employment of organic wastes as an alternative carbon source would account for a net reduction of the environmental impact of the industrial/civil activity which produced that waste. SCFAs have many applications in food, pharmaceutical, and cosmetics industries, and due to their functional groups, they are extremely useful for the chemical industry as precursors of reduced chemicals (esters, ketones, aldehydes, alcohols and alkanes) and the production of biofuels like methane and hydrogen, as well as biopolymers, such as polyhydroxyalkanoates (PHAs) (Strazzera et al. 2018). The most common process performed to produce SCFAs is anaerobic digestion (AD), which has dual benefits such as waste remediation and bioenergy production (Naresh Kumar et al. 2022). When AD is performed to mainly produce SCFAs, it is specifically referred to as acidogenic fermentation, and the operative conditions (pH, temperature, solids content, etc.) are set to favour the acidogenesis and prevent the formation of methane. These conditions also differ based on the characteristics of the waste. One of the crucial challenges in the optimization of the SCFA production is the increase of the concentration, which is usually around 15–30 g/L after the AD process, up to values that make the separation of the product from the medium convenient (Rebecchi et al. 2016). The storage and transport of SCFAs are generally safer and easier compared to biogas, given that the latter cannot be stored at atmospheric pressure; in addition, the added value of SCFAs is 50–130 $/ton (Fei et al. 2015), which

is higher than that of methane, 0.72 $/m^3 (Oleskowicz-Popiel et al. 2012). The literature regarding the use tannery sludge to produce SCFAs is scarce (the only study found was by Zhai et al. 2020), despite its rich organic matter and the high disposal costs of this waste, due to the presence of pathogenic microorganisms, heavy metal ions and other undesirable constituents (Liu et al. 2019). For this reason, in this study, the performance of the acidogenic fermentation of tannery sludge is compared with other type of organic wastes (waste activated sludge (WAS), sewage sludge, food waste, etc.) to assess whether this sludge is a viable substrate to produce SCFAs.

1.1 Anaerobic digestion (AD)

AD is one of the most widespread and well-known technologies in Europe for the treatment of a variety of organic wastes (Valentino et al. 2021). The main advantages of this procedure include the stabilization of the sludge, the reduction of total solids, and the recovery of bio-based products such as biogas and organic acids. The process is carried out by a complex community of bacteria and archaea, which transform, in absence of oxygen, the complex organic matter into products with an added value, such as SCFAs and biogas. The conversion of the substrate into these products can be summarized in four stages (Figure 1): hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In the hydrolysis phase, hydrolytic bacteria secrete extracellular enzymes that can convert carbohydrates, lipids, and proteins into sugars, long chain fatty acids (LCFAs), and amino acids (Li et al. 2011). These products can diffuse through the cell membranes of acidogenic microorganisms, where they are converted into SCFAs and other intermediate products (Meegoda et al. 2018). During acetogenesis, these SCFAs and other intermediates are converted into acetate, while hydrogen is being produced (Hansen et al.
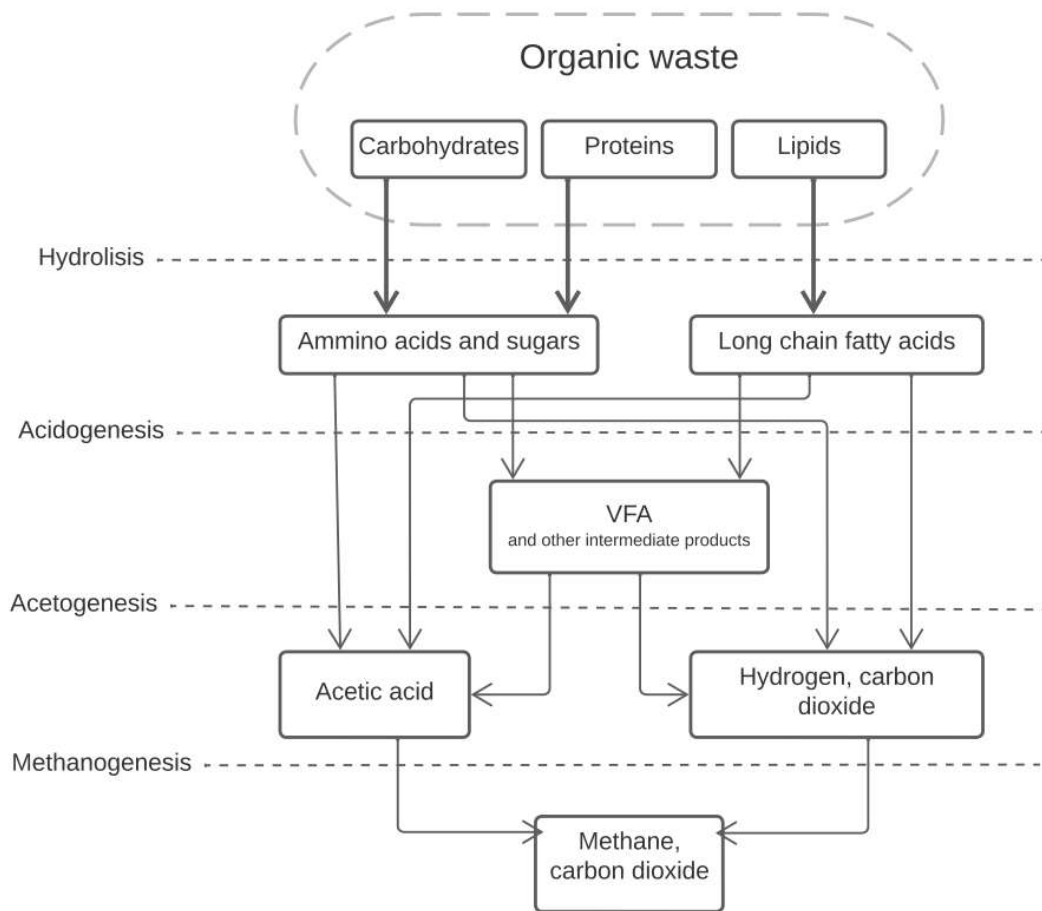
Figure 1 Simplified scheme of the anaerobic digestion pathways (Adapted from Batstone et al. 2002).

2013). During the final stage, methanogenesis, methane and carbon dioxide are produced, from a group of obligate anaerobic archaea. Based on the end products, the metabolic process of AD can be classified as: acidogenic fermentation, and methanation process (Fonseca et al. 2021, Ghysels et al. 2020). Nevertheless, the two fermentative stages are consecutive, and have different optimal operative conditions. Many authors performed the two steps into separate reactors; hence, producing SCFAs and hydrogen from the first stage and methane from the second stage (Micolucci et al. 2020, Valentino et al. 2021), with the possibility of using the digestate as fertilizer if the latter is free from toxic substances. The potential benefits of the anaerobic treatment of many organic residues

has been extensively explored and, up to now, only biogas production followed by methanation is the most applied scheme that found a specific market sector (Daryapurkar et al. 2001, Lefebvre et al. 2004, Banu et al. 2007, Mannucci et al. 2010). This technology allows to recover energy from organic waste, by reducing the amount of byproducts to be disposed of compared to the aerobic process (Mannucci et al. 2010).

1.2 Short chain fatty acids (SCFAs)

Short chain fatty acids (SCFAs) are a class of linear aliphatic mono-carboxylate compounds with a number of carbon atoms between two (acetic acid) and four (butyric and isobutyric acids). In some cases, medium chain fatty acids (MCFAs) can be also produced (from five to seven carbon atoms), even though is smaller amount compared to SCFAs. For simplicity reason, in this thesis, the acronym SCFAs is used to indicate all the organic acids, from acetic to heptanoic. The main physical and chemical characteristics of commercialized SCFA are summarized in Figure 2. These compounds have a high solubility in water, which tends to decrease with the increase of the number of C in the aliphatic chain. Nowadays, the commercial production of these compounds relies on the chemical synthesis using non-renewable materials such as raw petroleum (Volker et al. 2014) and pure sugars (glucose, xylose, etc.), which allows higher productivity with minimal side products (Feng et al. 2010, Zhu and Yang 2004). Both these methods are not environmentally sustainable: the first one uses fossil fuels, which are one of the main responsible for climate change, and the second one employs a carbon source that raises ethical concerns about the use of food and soil to produce chemicals (Zigová et al. 1999). The possibility of using organic-rich wastes as a carbon source would have the advantage of employing a process of the circular economy for bio-based
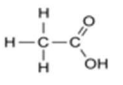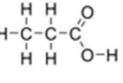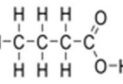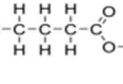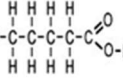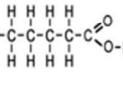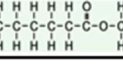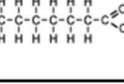
Common Name	Structural Formula	Mass (g*mol^{-1})	Density	Boiling Point	Solubility in Water (g*(dm3)$^{-1}$)	pKa a 25°C	Lewis Structure	Odour	Colour	Cost ($*ton^{-1})	Commercial Application
Acetic Acid	CH$_3$COOH	60.05	1.05	117.9	Fully miscible	4.76		Vinegar Smell	Colorless liquid	400-800	Food additive solvent, ester production, chemicals, polymers, dyes, adhesives.
Propionic Acid	CH$_3$CH$_2$COOH	74.08	0.933	140.99	Miscible	4.88		Pungent	Colorless liquid	1500- 1650	Animal and human food additive, chemical intermediate, solvent, flavoring agent.
Butyrric Acid	CH$_3$(CH$_2$)$_2$COOH	88.12	0.958	163.53	Miscible	4.82		Sweet smell	Colorless liquid	2000-2500	Esters used the food industry as aroma additive, food additive, flavoring, pharmaceuticals, animal feed supplement fishing bait additive.
Isobutirric Acid	CH$_3$-CH(CH3)-COOH	88.12	0.947	155	210	4.84		Fusty smell	Colorless liquid	1580-1600	It is used in the leather industry. Isobutyric, 2 - methyl butyric and isovaleric are considered as essential nutrients for many predominant rumen cellulolytic bacteria.
Valeric Acid	CH$_3$-CH$_2$-CH2-CH2-COOH	102.13	0.938	186.5	40	4.82		Unpleasant	Colorless liquid	1000-2500	It is used in the synthesis of its esters. It is also used in perfumes and cosmetics.
Isovaleric Acid	CH$_3$CH(CH$_3$)-CH2-COOH	102.13	0.925	176	25	4.78		Mould cheese smell	Clear colorless liquid	5000-2500	Isovaleric acid inhibits the synthesis of saturated fatty acids. It is a favorable carbon source for cell growth.
Caproic Acid	CH$_3$- CH$_2$CH$_2$-CH$_2$-CH2COOH	116.16	0.927	205	10	4.88		Sharp smell	Oily colorless liquid	2250-2250	Used in pharmaceuticals
Enanthic Acid	CH$_3$-CH$_2$- CH$_2$-CH$_2$-CH2-CH2-COOH	130.19	0.917	223	2.6	4.89		Unpleasant rancid	Oily colorless liquid	3000-4000	It is used in the preparation of esters, such as ethyl heptanoate. It is also used in drug industry.

Figure 2 Main physical and chemical characteristics, economic value, and applications of SCFAs.

production of bulk chemicals that is less impactful in terms of emissions and waste generation. The production of SCFAs from waste is obtained through acidogenic fermentation, which is an AD involving only the first two steps: hydrolysis and acidogenesis. Hydrolysis is the process in which complex organics polymers are broken into simpler organic monomers, while during acidogenesis these monomers ferment into SCFAs mainly (Lee et al. 2014). Both processes involve a complex consortium of obligate and facultative anaerobes, such as *Bacteriocides*, *Clostridia*, *Bifidobacteria*, *Streptococci*,

and *Enterobacteriaceae* (Weiland 2010). Although mixed microbial cultures may lead to lower yields in terms of SCFAs, they have several advantages, mainly, since non-sterile conditions are needed, the risk of damaging contamination is decreased (Bathia and Yang 2017). Several studies already employed different types of waste as their chosen substrate to maximize the production of SCFAs, employing different operational pH, temperature, retention time, organic loading rate, as well as the use of additives to affect the concentration, the yield, and the composition of SCFAs produced (Lee et al. 2014). SCFAs have many applications in several sectors such as the food industry, cosmetics, textiles, bio energy, and pharmaceuticals (Andersen et al. 2015; Baumann and Westermann 2016), and they can be utilized as building blocks for the chemical industry or as precursors of reduced chemicals and derivatives (esters, ketones, aldehydes, alcohols, and alkanes) in conventional organic chemistry (Valentino et al. 2021). Moreover, SCFAs produced from the anaerobic digestion of waste sludge has gained growing attention in making biodegradable plastics (PHA), or carbon source in biotreatment for wastewater (Wang et al., 2019; Xu et al., 2019). PHA is a family of naturally occurring biopolyesters synthesized by various microorganisms (Tan et al. 2014) which accumulate these substances as intracellular carbon and energy source, under nutrient-limited growth conditions (Lorini et al. 2020). The interest on PHA has progressively increased in recent times, since they are completely biodegradable, and they have thermoplastic properties comparable to traditional plastics (Lorini et al. 2022); moreover, the possibility of using a renewable carbon source to produce its precursors make it a sustainable material.

As previously mentioned, several factors influence the productivity of SCFAs from AD, namely pH, Temperature, total solids, and pretreatments which will all be dealt with in the next paragraphs.

1.2.1 Effect of pH

The pH value in the reactor is important for the production of SCFAs because most acidogenic microorganisms cannot survive in extremely acidic (pH 3) or alkaline (pH 12) environments (Liu et al. 2012). As explained by Lee et al. 2014, the range of pH values to produce SCFAs is between 5.25 and 11, but the optimal values depend on the type of waste used: for sludge alkaline pH is usually preferred, whereas for wastewaters neutral and acidic conditions tend to favour the production of SCFAs. Besides, the alkaline environment is not conducive to methanogenesis, thus preventing the consumption of the produced SCFAs for methane formation (Zhang et al. 2009a). Moreover, the growing concentration of SCFAs can lead to a drop in the pH value, which can be neutralized by the alkalinity, maintaining a stable pH. Many authors investigated the relation between pH and alkalinity with SCFA production. Garcia-Aguirre et al., 2017 evaluated the effects of pH and temperature on SCFA production efficiency from municipal solid waste, and the highest SCFA production (8.3 g COD/L) was achieved under alkaline pH and mesophilic condition. Cheah et al. (2019) used food waste as substrate and found that, when the operative pH was changed from 6 to 7, the SCFA production was boosted and a higher production of propionic and valeric acids was recorded. Valentino et al. (2021) studied the anaerobic digestion of food waste, and the higher pH used (6.6), compared to previous studies (5.0-5.5), was one of the factors that lead to a higher concentration of SCFAs in the stream. The pH also influences the type of SCFAs produced (Bengtsson et al. 2009). Atasoy et al. (2019) found that at neutral pH values, acetic acid was produced

at the highest levels, while at acidic values a mix of acetic and butyric acid was dominant; the productivity was also investigated, and high pH values lead to higher SCFAs production.

1.2.2 Effect of temperature

Temperature affects the growth of microorganisms, the activities of enzymes, and the hydrolysis of particulate organic matters to soluble substances (Kim et al. 2003), which are all enhanced with the increase in temperature. However, anaerobic microorganisms in thermophilic conditions are more sensitive to environmental changes than in a mesophilic process (Kim et al. 2006). Valentino et al. (2022), using a mixture of the organic fraction of municipal solid waste (OFMSW) and waste activated sludge (WAS), studied the production of SCFAs and biogas under two temperature conditions, obtaining similar results in the mesophilic (37°C, 20 g COD_{SCFA}/L) and thermophilic conditions (55°C, 16.5 – 31.6 g COD_{SCFA}/L). Through the SCFA levels were generally higher at 55°C, frequent fluctuations were observed, resulting in a process more difficult to control and less stable compared to the mesophilic condition. The solubilization of the COD was also reported to be higher in the thermophilic process, but a non-negligible fraction of the sCOD remains unconverted into SCFAs. A wider range of temperature was investigated by Soomro et al. (2020); in this study, the OFMSW was also separated into food and paper fraction, and the three different substrates were subject to four different temperatures of AD (25-37-42-52°C). The best result was given by the mixed waste, with 21.5 g COD/L at 42°C, while food fraction had the best result at 37°C (15.1 g COD/L) and paper fraction at 52°C (10.2 g COD/L).

These results show that the optimal temperature for the highest SCFAs production depends on the substrate properties (alkalinity, organic matter complexity, presence of

other chemicals…), and that the enhanced kinetics and solubilization provided by higher temperatures do not ensure higher yields.

1.2.3 Effect of total solids content (TS)

TS content is an important attribute of anaerobic digestion. Higher TS allow the use of a smaller digester volume, which reduces the initial costs. However, higher TS and/or TSS content in tannery wastewater reduce mass transfer, settling rates (Song et al. 2000), and may alter other rheological properties of the medium, such as the viscosity (Battista et al. 2018); consequently, this can affect the microbial communities, their activity, and metabolic pathways. When the TS content is less than 15% (w/w) we refer to wet digestion (Karthikeyan et al. 2013), characterized by a liquid medium, which allows homogeneity in the bioreactor, full access of the degrading microorganisms to the substrate, and dilution of inhibitory products (Pommier et al. 2007). The same mechanisms should be valid for the fermentation of tannery sludge to produce SCFAs. Motte et al. (2013) compared the substate conversion into fermentative end-products (mainly hydrogen, acetic and butyric acids) at different TS contents (10 to 33%) and found that the conversion yield decreased by 30% when switching from wet to dry conditions. Lim et al. (2008) studied the effect of the organic loading rate (OLR) on acidogenesis of food waste in a semi-continuous reactor, obtaining 14.0, 24.0, and 30 g SCFA/L with an OLR of 5.0, 9.0 and 13.0 g TS/(L*d), respectively. Although the higher concentration was obtained with an OLR of 13.0 g TS/(L*d), yield was lower compared to the other tests. As explained by Liu et al. (2018b), the SCFAs production increased with the increasing TS content up to a point, when a higher TS content inhibited further degradation, leading to a lower conversion efficiency of SCFAs production.

1.2.4 Pretreatment of sludge for AD

Another aspect investigated was the application of a pretreatment to support the initial hydrolysis of the tannery sludge, given its high amount of proteins and organic macromolecules; in addition, these molecular structures lead to a high concentration of ammonia, which inhibits the bacteria and the hydrolysis rate of organics (Zhai et al. 2020). With the application of a pretreatment, that might be mechanical, chemical, thermal, or enzymatic, it is possible to disintegrate extracellular polymeric substances (EPS), disrupt microbial cells to release soluble organics, and improve sludge biodegradability (Liu et al. 2017). Various studies on the application of a combined pretreatment with microwave irradiation and hydrogen peroxide have been reported, using sewage sludge from wastewater treatment plants as main substrate (Eskicioglu et al. 2008, Liu et al. 2017, Liu et al. 2018a, Ambrose et al. 2020). Xiao et al. (2012) reported that the soluble chemical oxygen demand (sCOD) was increased by 30.52 times after MW-H_2O_2 treatment. However, these studies are mainly concerned with the methane production from AD, while no article about the use of the combined pretreatment to obtain SCFAs has been found. The microwave irradiation, as an alternative technology to conventional thermal heating, has the advantages of heating rapidly, uniformly, and with high energy efficiency (Tyagi and Lo 2013). Microwave pre-treatment at higher temperatures between 110 and 175 °C is considered to be very efficient for sludge solubilization (Liu et al. 2017); however, pre-treatments conducted at temperatures lower than boiling point are as well applicable (<100°) (Kuglarz et al. 2013) and have the potential of enhancing sludge solubilization and anaerobic digestion under more acceptable conditions (and presumably in a more economically sustainable matter) from the operative point of view (Eskicioglu et al. 2008). Hydrogen peroxide (H_2O_2) is a strong oxidant that produces OH• radicals

(Eskicioglu et al., 2008), as well as superoxide radicals $O_2^{\bullet-}$ (Hayyan et al. 2016), which are toxic for most of the microorganisms and lead to the destruction of the cellular structures, and the oxidation of complex organic matter into simpler and more soluble substances. Eskicioglu et al. 2008 found that the dissociation of H_2O_2 into its radicals is not rapid in case of wastewater with high refractory compounds, and it can be accelerated by microwave irradiation. According to Liu et al. 2017, high H_2O_2 dosages can have an inhibitory effect on the growth of the bacteria, as well as generate refractory compounds; different dosages have been tested, based on the concentration of the total solids (TS): 0.2-0.6-1.0 gH_2O_2/gTS; the dosage of 0.2 gH_2O_2/gTS has been reported to be the more appropriate to enhance hydrolysis in the AD.

Another commonly employed oxidation technique is ozonation, which facilitates solubilisation and mineralisation of sludge particles with the release of biodegradable and non-biodegradable constituents (Chiavola et al. 2021). During sludge ozonation, ozone decomposes itself into radicals and reacts with the whole matter (Böhler & Siegrist 2004), so it must be generated on site. A study by Tunkay et al. (2022) investigated the impact on methanogenic consortium of the pretreatment with different ozone doses (0.03-0.06 and 0.09 g O_3/g TSS), with the objective of enhance sludge solubilization and overcome the rate-limiting hydrolysis step of the AD. The results showed that the optimal dosage was 0.06 g O_3/g TSS, which resulted in a 39% reduction of the TS compared to the non-ozonated sludge. Cesaro & Belgiorno (2020) used ozonation pretreatment on the organic fraction of municipal solid waste, dosing 0.16 g O_3/g TS and improving the final production of methane up to 95%, with a positive net energy gain, with respect to either electrical or thermal energy. However, ozone pre-treatment requires higher investment

and operational costs as compared to other techniques for sludge reduction (Wang et al. 2018).

1.3 The tannery sludge

Leather processing, as described by Giaccherini (2017), is a complex combination of mechanical and chemical procedures, which can be divided into three main phases: acquisition and pre-treatment of raw animal hides, treatment of the hides with a tanning agent, and drying and shining the hides before sending them to the manufacturers. The tanning can be done using two different tanning agents, chrome or vegetable tanning, where chrome tanning remains the principal method, accounting for 80-90% of the leather produced worldwide (Swartz et al. 2017). Tannery sludge is originated from the treatment of wastewater; the tanning process consumes more than 100 L of water and about 2 kg of chemicals per square meter of final product and it requires more than 1 ton of oil equivalent per 1000 m^2 of final product as energy supply (UNIC, 2010). The wastewater is characterised by high organic load in terms of COD, organic nitrogen, sulphur, high levels of suspended solids, heavy metals, and other chemicals (Mannucci et al. 2010), consequently, it is normally collected and treated in centralised treatment plants. The common treatment line is represented in Figure 3; after the preliminary treatment, the equalization tank is aerated to keep the particulate matter in suspension and oxidize the
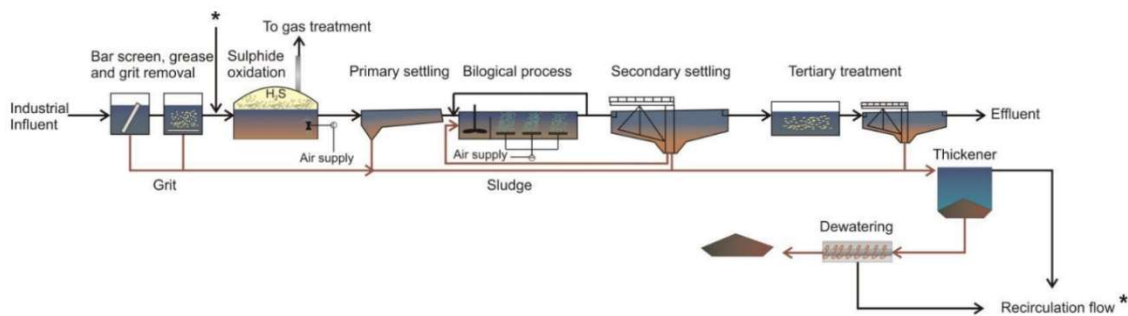

Figure 3 Common tannery wastewater treatment process scheme (Giaccherini 2017).

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sulphide. For this reason, the reactor must be closed, allowing the produced gas to be collected and treated before discharge (Giaccherini 2017). The rest of the line is analogue to a treatment process with activated sludge. In certain cases, tertiary treatment of oxidation (with H_2O_2 or ozone) is required to mineralize the recalcitrant COD and meet the discharge limits (Giaccherini 2017). At present times, tannery sludge is mainly disposed of in landfills (Alibardi and Cossu 2016), as for the Italian legislation that classifies this sludge as a special non-hazardous residue (Ronchi Decree, 2/97) and sets its current destination is a second-class type B controlled landfill (D. Lgs. 04/06).

However, other methods have been explored, one example is composting (Santos et al. 2011), in which the organic compounds are transformed into nutrients, while the Cr can be absorbed and concentrated in plants (Rani et al. 2017).

The main risks associated with this sludge are the high levels of chromium (Cr), as well as several organic compounds harmful to both the environment and human health; the removal of these pollutants remains very challenging, and this prevents other possible uses of this waste (Liu et al. 2019). Moreover, the sludge management costs account for around 30-40% of the overall budget of a tannery wastewater treatment plant (Akyol et al. 2015), so its valorization might be an appealing alternative to the simple disposal. Many potential products can be derived from tannery wastewater/sludge, such as organic acids, metals, industrial enzymes, biofuels, fertilizer, and compost (Mpofu et al. 2021). Despite this, the studies dealing with its potential valorization are scarce (Bautista et al. 2015) and mostly focus on anaerobic digestion (AD) of wastewater for biogas production for onsite uses as electricity generation or heating purposes (Mpofu et al. 2021).

2. Thesis objective

The purpose of this work is to provide an alternative to landfill disposal of tannery sludge, optimizing AD conditions to maximize the production of SCFAs via acidogenic fermentation up to concentration and yield values that allow the technical and economic feasibility of their extraction. The main advantages of this biological process are the recovery of added value chemicals, the reduction of the waste to dispose of, and the implementation of a more environmentally friendly process compared to the present one. A comprehensive acidogenic fermentation batch tests assay has been developed and discussed in this study, where the effect of temperature, pH, total solids (TS) concentration, and microwave and H_2O_2 pretreatment has been investigated on SCFAs production (rate, yield, and concentration) and distribution.

In particular, the chosen values of these parameters were:

- pH: as no information regarding the optimal range of values for the AD of tannery sludge has been found, in this work we have investigated four initial pH, namely 5-7-9, and 11.

- Temperature: the acidogens, which are the responsible for the SCFAs production, include both mesophilic (25–45°C) and thermophilic (45–65°C) bacteria (Patel et al. 2021), consequently the production of SCFAs from tannery sludge has been tested in mesophilic (40 ± 1°C) and thermophilic (55 ± 1°C) environments, to determine the best temperature condition to maximize the yield.

- Total solids: the TS contents tested in this work were 8% and 12% (w/w). Higher values of TS are associated with higher values of volatile solids (VS), so an increase in the production of SCFAs is expected, while the effect on the yield (g SCFA/g $VS_{(0)}$) are not foreseeable.

- MW-H_2O_2 pretreatment: the correct dosage of oxygen peroxide should enhance hydrolysis and avoid the presence of residual H_2O_2; a dosage of 0.2 g H_2O_2/g TS was applied, based on the results obtained by Liu et al. 2017. The microwave irradiation was performed to thermal pretreat the sludge up to 90°C (the detailed procedure is described subsequently).

3. Materials and methods

3.1 Substrate

The tannery sludge for the experiments was taken from the WWTP of Montebelluno Vicentino, in the province of Vicenza, Italy, which collect the wastewater of several tanneries in the area. The waste was a mixture of primary and secondary sludge, had a concentration of TS of 84%, reached with a process of dehydration performed in the downstream of the plant. This WWTP treats about 10000 m^3/d of industrial wastewater produced by 23 tannery plants. Tannery wastewaters are subjected to primary treatment and sedimentation (accomplished inside both tanneries and WWTP), and secondary biological treatment (anoxic/aerobic process) used for biochemical oxygen demand (BOD) and nitrogen removal from the primary clarified effluent.

3.2 Batch tests preparation

The different combination of initial conditions (TS, pH, temperature, pretreatment) were performed using 8 bottles, four in mesophilic condition and four in thermophilic condition. For each of these condition, four different initial pH values have been tested. In total, three different fermentation rounds have been performed. The summary of the operative condition of each test is shown in Table 1.

Table 1 Summary of the operative conditions of each test.

Test name	M8	M12	M8-P	T8	T12	T8-P
Temperature (°C)	40	40	40	55	55	55
Solids (g TS/L)	80	120	80	80	120	80
Initial pH	5-11	5-11	5-11	5-11	5-11	5-11
MW-H_2O_2	no	no	yes	no	no	yes

Figure 4 Preparation of the bottles used to perform the acidogenic fermentation after pretreatment of the sludge.

The 8% and 12% TS were prepared by diluting 20 g and 30 g, respectively, of the dried sludge with 200 mL of tap water in the bottles. The reactors consisted of glass bottles with a total volume of 250 mL, sealed with a cap with a silicon plug to maintain the anaerobic condition and to be able to withdraw the samples. The pH of each solution was adjusted using a pH-meter while adding sulfuric acid (H_2SO_4) and Sodium hydroxide (NaOH) to tap water, before adding the sludge, to the following pH values: 5.0, 7.0, 9.0, and 11.0. The combined MW-H_2O_2 pretreatment was performed based on the strategy proposed by Liu et al. 2017; 2018a and Ambrose et al. 2020, with some adaptations. After reaching the required pH, each bottle was first prepared with 20 g of TS in 192 mL of water (to leave some volume for the addition of H_2O_2) and heated up to 80°C with a microwave oven set at 600 W for 10 minutes. Every 1,5 min the treatment was stopped to avoid water loss by evaporation and to allow manual mixing of the sludge for a more

homogeneous condition. After that, the bottles were allowed to cool to room temperature and the H_2O_2 pretreatment was performed, with the chosen amount of 0.2 g H_2O_2/g TS, using a solution of H_2O_2 at 35% (8,5 ml were added in each bottle). The bottles were left for 40 minutes to rest allowing the H_2O_2 to react and then were heated again with the MW to 90°C, to avoid reaching boiling temperature, with the same method described before (Figure 4). When the bottles cooled down, an inoculum of fresh tannery sludge was added to start the fermentation, as microbial community present on the sludge had been compromised during the pretreatment.

3.3 Batch fermentation test

The bottles were put in an oven for the mesophilic (40 ± 1°C) condition, and in thermostatic bath for the thermophilic (55 ± 1°C) condition (Figure 5). The bottles were

Figure 5 Thermostatic bath used to perform the thermophilic fermentation.

manually mixed several times a day. A sample was withdrawn 3 times a week, to measure the production of SCFAs, the pH trend, the soluble COD trend, and the nutrients concentration (N- NH_4^+, P- PO_4^{3-}). The samples had a volume of 5 mL and were taken with a syringe through a rubber septum, allowing the maintenance of anaerobic conditions; before the sampling, the bottles were manually mixed and after 10 min the supernatant was withdrawn, around 2 cm from the surface. Immediately after, the samples were centrifuged at 4300 rpm for 5 min to separate the suspended solids (TSS) from the supernatant, which was subsequently filtered with a 0.22 μm acetate cellulose syringe filter. The fermentations were stopped once a plateau of the concentration of SCFAs (measured as mg COD/L) was observed.

3.4 Analytical methods

Analyses were conducted according to Standard Methods (APHA 2005) for the TKN, N- NH_4^+ total phosphorus, P-PO_4^{3-}, VS, TS, Cr(VI) and COD. Analyses for the initial characterisation of the tannery sludge (COD, TKN, total phosphorus, TS, and VS) were performed after drying the sample for 48h at 105°C.

3.4.1 SCFA analysis

Volatile fatty acids were determined using an Agilent 6890 N gas chromatograph (Figure 6) equipped with a flame ionization detector (FID) (T = 250 °C). Samples were analysed 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 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 200°C (10 °C/min), and a post-run phase at 220°C. The target organic acids were: acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid,

Figure 6 Gas chromatograph used for SCFAs determination.

caproic acid, isocaproic acid, and heptanoic acid. For the calibration of the instrument, three different dilution of a standard mix solution (containing the target SCFA) were prepared: as is, 1:2, and 1:4. A fixed amount of internal standard (2-etilbutyrric acid) was also added. Four points in total were taken for the calibration, three from the standard dilutions, and the axes origin; the calibration was considered successful with a $R^2 > 0.995$.

3.4.2 Chemical oxygen demand (COD)

In this study, COD is referred to the sludge before the fermentation tests, while the sCOD is determined on the samples taken from the bottles at different stages of the acidogenic fermentation. The method provides the pulverization of the sample with a mortar, and the oxidation with potassium dichromate ($K_2Cr_2O_7$) in presence of concentrated sulfuric acid (H_2SO_4), silver sulfate (Ag_2SO_4) as catalyst solution, and mercury sulfate ($HgSO_4$) to avoid the interference of the chlorides, oxidable by the potassium dichromate. For the sample digestion, a series of vessel made of *Teflon®* with a certain amount of dried sludge and the reagents were placed in a mineralizer (Figure 7) with a two-step process, the first at 175°C and the second at 50°C for 1 h in total. During this, the organic carbon is

Figure 7 Mineralizer used for the digestion of the dried sludge.

oxidized, and the potassium dichromate in excess is quantified by titration with ammonium sulphate and iron (FAS solution) using ferroin as indicator. The equation to quantify the COD as mg O_2/g TS is:

$$COD = \frac{(B - C) * \left(\frac{V * N}{T}\right) * 8}{P}$$

where:

B: volume of FAS used for blank titration (mL)

C: volume of FAS used for sample titration (mL)

N: normality of dichromate

V: volume of $K_2Cr_2O_7$ used (mL)

T: volume of FAS used to quantify the titrant concentration (mL)

8: weight of the oxygen (g O_2/eq)

3.4.3 Soluble chemical oxygen demand (sCOD)

The soluble chemical oxygen demand (sCOD) is a fraction of the COD that quantifies the soluble organics (Zhang et al. 2009b). Its measure provides information on the fraction of soluble organics that have been converted into SCFAs. The analysis was performed with colorimetric method, using a spectrophotometer Analytikjena Spekol 1500 (Figure

Figure 8 Spectrophotometer used for the analysis.

8). A certain amount of sample is placed in a test tube, then an oxidizing solution ($K_2Cr_2O_7$) and a catalyst solution ($H_2SO_4 + Ag_2SO_4 + HgSO_4$) are added. The test tubes are placed in an oven at 150°C for 2 h, then the absorbance at 600 nm wavelength was measured to define the concentration of sCOD (mg O_2/L). The amount of sCOD was determined with the following equation:

$$sCOD = sCOD_{mis} * D.F.$$

where:

$sCOD_{mis}$: mean value obtained with the spectrophotometric analysis

D.F.: dilution factor of the sample, before the addition of the reagents

3.4.4 Total solids/Volatile solids

TS were determined by first weighing the empty crucible (tare), and then the crucible with the sample (wet weight); the latter is then put in the oven for 48 h at 105°C, to dehydrate the sample, and then is weighted to determinate the dry weight. The equation used for the quantification of TS as g TS/Kg$_{(sludge)}$ was:

$$TS = \frac{W2 - W0}{W1 - W0} * 1000$$

where:

W0: weight of the empty crucible

W1: wet weight of the crucible with the sample

W2: dry weight of the crucible with the sample

Figure 9 Oven and muffle used to dehydrate/incinerate wet sludge.

For the quantification of VS, the same sample resulting from the previous procedure (W2) must be incinerated in the muffle (Figure 9) for 24h at 550°C. The crucible with the ashes resulting from the incineration is weighted (W3), and the VS amount is given by the equation:

$$TVS = \frac{P2 - P3}{P1 - P0} * 1000$$

3.4.5 TKN / Ammonia nitrogen

The TKN method is used to determinate the total amount of initial nitrogen in the sludge (TKN = N_{ORG} + N-NH$_3$ + N-NH$_4^+$). The sample is pulverized, placed in a vessel made of *Teflon®* and then it goes under acidic digestion with concentrated sulfuric acid and potassium peroxidisulfate ($K_2S_2O_8$) in the mineralizer in two steps; the first step occurs at 200°C for 85 min, the second at 200°C for 95 min. At the end of the first step, the vessel is opened and hydrogen peroxide is added (H_2O_2); after a few minutes the vessel is closed again for the second step. The resulting solution is filtered with a black ribbon filter, then the ammonia is steam distilled (the distiller used is a VELP SCIENTIFICA UDK 129, Figure 10) to avoid the loss due to volatilization. The spectrophotometric analysis is based on the reaction of the ammonium ion with the Nessler's reactive, which create a yellowish solution, and the absorbance is read at 410 nm wavelength. The value of the TKN (g N/Kg TS) is given by the following equation:

$$TKN = \frac{m * ABS * D.F.* V}{P}$$

where:

m: slope of the straight line resulting from the calibration

ABS: absorbance obtained by the spectrophotometric analysis

D.F.: dilution factor before the addition of the Nessler's reactive

V: volume of the flask where the digested was diluted (0,25 L)

P: weight of the dry sample (g TS)

Figure 10 Distiller used for the TKN analysis.

The ammonia nitrogen was analysed on the samples taken from the fermentation tests; the procedure is the same described for the TKN starting from the distillation of the ammonia to the spectrophotometric analysis, and the equation for the ammonia value (as mg (N-NH$_4^+$)/L) is the following:

$$N - NH_4^+ = m * ABS * D.F.$$

where:

m: slope of the straight line resulting from the calibration

ABS: absorbance obtained by the spectrophotometric analysis

D.F.: dilution factor operated before addition of the Nessler's reactive

3.4.6 Total phosphorus / Orthophosphate

For the total phosphorous, the procedure is analogue to the one reported for the TKN (pulverization, mineralization…), but the objective here is to transform the whole phosphorous present in orthophosphate; after filtration with black ribbon filter, the determination is based on the reaction of orthophosphate ions with ammonium molybdate and potassium antimonyl-tartarate (mixed reagent), with the formation of a heteropolyacid which is subsequently reduced by ascorbic acid to molybdenum blue, an intensely colored compound. Spectrophotometric determination is performed, obtaining the absorbance of the sample at a wavelength of 710 nm. The equation that gives the value of the total phosphorous (g P/Kg TS) is:

$$P_{TOT} = m * ABS * D.F. * V * P$$

where:

m: slope of the straight line resulting from the calibration

ABS: absorbance obtained by the spectrophotometric analysis

D.F.: dilution factor before adding the mixed reagent

V: volume of the flask where the digested was diluted (0,25 L)

P: weight of the dry sample (g TS)

The orthophosphate ion was quantified on the samples taken from the fermentation tests; the procedure is the same described for the total phosphorous starting from the filtration phase, which is already performed during the procedure of sampling with a 0.22 μm acetate cellulose syringe filter, consequently the use of black ribbon filter is not required. The equation to quantify the orthophosphate (as mg (P-PO$_4$$^{3-}$) is:

$$[PO_4^{3-}] = m * D.F.* ABS$$

where:

m: slope of the straight line resulting from the calibration

ABS: absorbance obtained by the spectrophotometric analysis

D.F.: dilution factor operated before adding the mixed reagent

3.4.7 pH

The pH was monitored with each sampling. The pH meter (Figure 11) was calibrated with 3 solutions with known pH (4, 7, 10), then the sample was measured, after the filtration. The instrument was also used to set the initial pH of each batch test.

Figure 11 pH meter used for the analysis.

3.4.8 Hexavalent chromium [Cr(VI)]

Hexavalent Chromium Cr(VI) was analyzed in the liquid phase at the end of each tests to quantify any possible Cr(VI) release. The analyses were performed by an outside lab. The hexavalent chromium was determined calorimetrically by reaction with diphenylcarbazide in acid solution (pH 1.6-2.2), reached adding a certain amount of H_2SO_4. The adsorption was measured at 540 nm wavelength.

4 Results and discussion

4.1 Characterization of the initial matrix

The analyses performed on tannery sludge, obtained at the end of the WWTP process (Figure 12), are reported in Table 2. There results, except for TS, refer to the dry matrix, obtained after a 48 h dehydration in the oven.

Table 2 Chemical characterization of the tannery sludge

Parameter	Unit	Value
TS	g/Kg	830 ± 14
VS	g/Kg	590 ± 4
COD	$gO_2/KgTS$	793 ± 18
TKN	gN/KgTS	32.8 ± 0.9
P_{TOT}	gP/KgTS	7.9 ± 0.4

This waste, by its nature, is subject to a certain variability due to the different raw materials used and the tanning process itself. This can result in different characteristics of the sludge in both spatial and temporal terms; as an example, in the study of Alibardi

Figure 12 Dry sludge collected from the Montebelluno Vicentino WWTP

and Cossu (2016), the dried tannery sludge collected from the WWTP had a higher TS (92%) and VS (74%) content, a TKN of 69 g N/Kg TS, and a P_{TOT} concentration of 3.8 g P/Kg TS. The relatively high levels of COD and TKN derived from the tannery wastewater (Mannucci et al. 2010), which is rich in these chemical species; therefore, the amount of total phosphorus in tannery wastewater is very low (Zhao and Chen 2019), and this is reflected in the resulting sludge.

4.2 Effect of the initial pH on the tannery sludge fermentation

The monitoring of the pH revealed that in all the performed tests, approximately after 5 days from the beginning of the fermentation, the pH values converged to 7 (Figure 13 report the tends of pH as mean value of each condition performed). For this reason, no

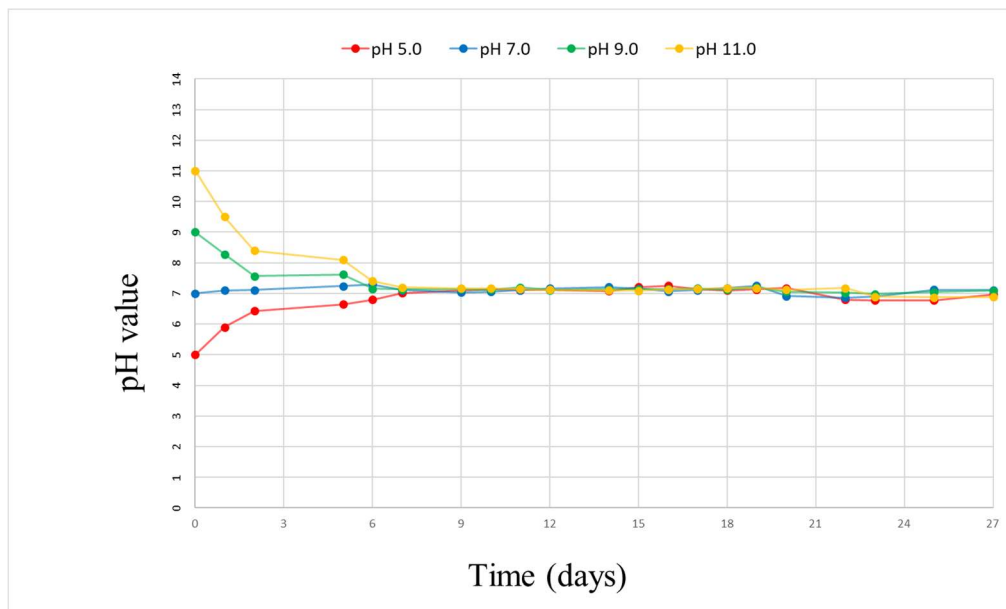
Figure 13 Mean pH trends observed in all tests performed.

significant influence of the pH on the acidification performances was observed, and no consideration can be made about the initial pH to distinguish the tests. This highlights the

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ability of the tannery sludge to act as a buffer and maintain a pH value around 7 with relatively high concentration of SCFAs. This is due to the presence of lime, which is used in the liming and unhairing stages of the processing of the raw hide, and in the tertiary treatment of the wastewater (Giaccherini 2017). This characteristic is noteworthy for future large-scale application, since an external intervention to correct the pH will not be necessary. As the concentration of acids grow, the alkalinity provided by the lime avoids the drop of the pH to acidic values. Moreover, neutral pH (around 7) is considered by many studies the most suitable for the SCFAs production (Liu et al. 2018b).

4.3 Production of SCFAs over time

The graphs of the mesophilic tests (Figure 14) all show a similar behaviour of a fast initial SCFAs concentration growth followed by a plateau. The M8 series reached the plateau after day 9, while the series M12 reached the plateau on day 15. In batch test M8 the specific rate had a gradual slowdown before reaching the plateau after approximately 10 days. For M8-P, the fermentation trend is similar to M12, with the plateau reached approximately on day 15, but the values of specific rate of production are higher, especially in the initial stages of the fermentation. These results prove that the initial TS influenced the acidification process, which is slower for the M12 compared to M8; this might be explained by the higher viscosity, due to the higher TS, which can negatively affect the fermentation (Battista et al. 2018). The analysis of the required time for maximum SCFAs concentration is a measurement of the total exploitation potential of this sludge in terms of organic matter acidification and the future development on industrial-scale as a continuous process (CSTR) by selecting the appropriate hydraulic retention time (HRT) based on the desired production. As for the maximum concentration of SCFAs archived, the M8 test performed between 9.5-10.3 g COD_{SCFA}/L. Higher results

were obtained by the M12 series, where the concentration was in the range of 14.6-15.8 g COD_{SCFA}/L, similar to M8-P which yielded slightly higher maximum concentration (16.8-17.7 g COD_{SCFA}/L) slightly higher. The pretreated sludge performed the best in terms of concentration of SCFAs, proving that the microwave combined with partial oxidation of the organic matter can affect the acidification; when compared with M8, the production was almost doubled.

For the thermophilic series (Figure 15), the trends of T8 and T12 are analogue to the mesophilic condition, even though the plateau was reached sooner due to the faster kinetic typical of the higher temperatures (Vidal-Antich et al. 2021). In T8 the plateau was abruptly reached on day 8, while T12 was characterized by a more gradual slowdown of the specific rate of production until reaching the plateau on day 12, again due to the influence of TS. In the series T8-P the trend appears different from both the other thermophilic condition and the M8-P, as the SCFAs production was continuously increasing until the very final days (24 to 27), when it finally slowed down, but without reaching an evident plateau. The temperature (Kim et al. 2003) and the MW-H_2O_2 pretreatment (Xiao et al. 2012) both contributed to enhance the solubilisation of organic matter and triggered a long process of acidogenic fermentation. However, an HRT of more than 20 days is normally difficult to manage in a continuous process from the economic point of view, despite the high production potential of this operative conditions. The concentration peaks of the T8 and the T12 were 6.7-7.4 g COD_{SCFA}/L and 13.9-15.3 g COD_{SCFA}/L respectively, which are lower compared to the mesophilic condition. As reported by Mpofu et al. (2021), conventionally, mesophilic AD at 35-40°C is preferable to thermophilic AD when treating tannery wastewater, in fact it may exacerbate inhibition instead of improving reaction kinetics and process efficiency. The series T8-P reached
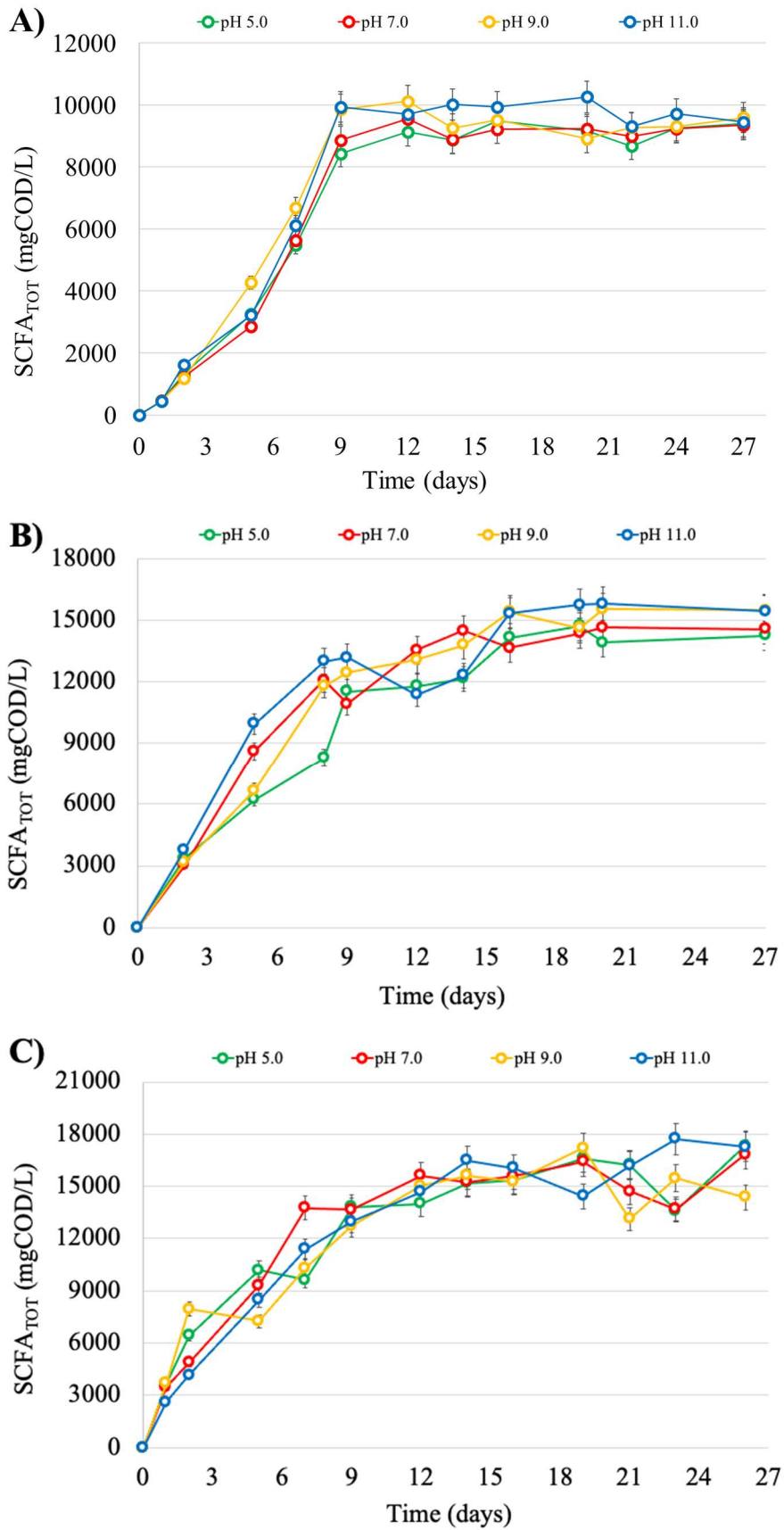
Figure 14 SCFAs concentration trends (mgCOD/L) in the mesophilic batch test M8 (A); mesophilic batch test M12 (B); mesophilic batch test M8-P (C).
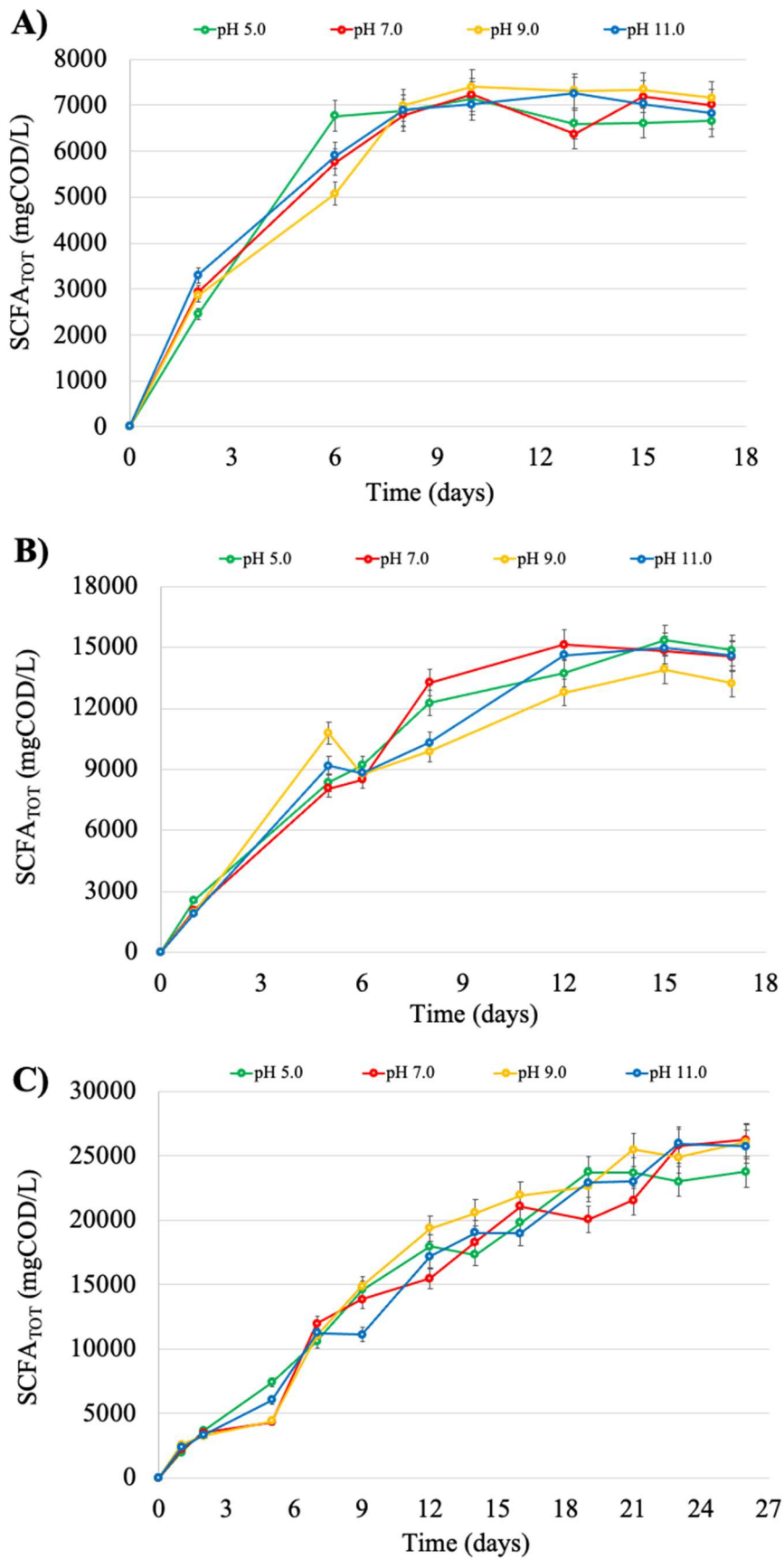
Figure 15 SCFAs concentration trends (mgCOD/L) in the thermophilic batch test T8 (A); thermophilic batch test T12 (B); thermophilic batch test T8-P (C).

concentrations between 23.7-26.2 g COD_{SCFA}/L, much higher than M8-P (16.8-17.7 g COD_{SCFA}/L), highlighting the combined effects of high temperatures and the MW-H_2O_2 pretreatment on the SCFAs production. Overall, the mesophilic condition seems more appealing for a future industrial-scale development for the SCFAs production process from tannery sludge, also taking into account the economic drawback of the thermophilic condition related to heat requirements.

The literature regarding the production of SCFAs from tannery sludge is scarce, so a comparison can be assessed with municipal sludge related experiments, since both derived from a wastewater biologically treated in WWTPs. Lorini et al. (2022) tested the production of SCFA from thickened WAS in three different conditions, obtaining 5.2 ± 0.3 g COD_{SCFA}/L in mesophilic temperature, 7.7 ± 0.3 g COD_{SCFA}/L in thermophilic temperatures, and 8.8 ± 0.1 g COD_{SCFA}/L in thermophilic temperature with thermal pretreatment (70°C for 48 h). These results were obtained with a solids content of 30 g TS/Kg_{WAS} in 8-9 days (HRT), which is a time closer to the M8-T8 than the M12-T12 of this study; this is a confirmation that the lower TS reduce the time required for complete acidogenic fermentation. A mixture of WAS and OFMSW was used by Valentino et al. (2019) in an AD process (CSTR) to produce SCFA, obtaining 20 g COD_{SCFA}/L in mesophilic conditions (37°C); in thermophilic conditions (55°C) the concentrations were generally higher, but widely variable over the course of the process, in a range from 16.5 to 31.6 g COD_{SCFA}/L, resulting in a more difficult fermentation control. Morgan-Sagastume et al. (2010) conducted a semi-continuous fermentation of WAS pretreated via Cambi (high pressure and temperature) process, adopting an operative temperature of 42°C and obtaining 19.9 ± 1.9 g COD_{SCFA}/L from the mixed primary-WAS and 18.5 ± 2.2 g COD_{SCFA}/L from the municipal WAS. These values are between those obtained in

this study by the M8-P (16.8-17.7 g COD$_{SCFA}$/L) and the T8-P (23.7-26.2 g COD$_{SCFA}$/L). In Morgan-Sagastume et al. (2015) the thickened WAS fed four acidogenic fermentation batches, without pH control and pretreatments, conducted at different operative temperatures; no statistically relevant differences were found regard the maximum SCFA productions (between 6 ± 0.9 and 9.4 ± 1.6 g COD$_{SCFA}$/L), archived in 4.5 days at 35°C and in7 days at 55°C. In general, we can observe that when the substrate is composed only of WAS, the concentrations of SCFAs obtained are lower than the mixed substrates (primary sludge/OFMSW and WAS); in all studies, the production was further enhanced by performing pretreatments. The effect of the temperature seems to be inconsistent and dependent on the characteristics of the sludge and the process itself.

4.4 SCFA/sCOD ratio

The amount of sCOD that converted into SCFAs indicates whether the concentration of soluble organics can affect the conversion efficiency of acidogenic fermentation, and if the enhanced solubilization given by temperature and pretreatments can drive to higher production of acids. The highest value of sCOD, at the end of the fermentation, was archived by T8-P (34.1 g O$_2$/L), as expected due to the application of the MW-H$_2$O$_2$ pretreatment combined with the thermophilic temperature. T8 had the lowest value (12.6 g O$_2$/L), confirming again to be the worst condition studied. Figure 16 shows the SCFA/sCOD ratios (COD/COD); these results were calculated on the plateau reached in each acidogenic fermentations, where the higher SCFAs concentrations were registered. The mesophilic tests (Figure 16A) all show similar results, around the value of 0.7 COD/COD, so solids content and pretreatment don't seem to have an influence on this parameter.

Figure 16 SCFA/CODsol ratio (COD/COD) of the mesophilic batch tests M8, M12, and M8-P under initial pH 5, 7, 9, and 11 (A); thermophilic batch tests T8, T12, and T8-P under initial pH 5, 7, 9 and 11 (B).

The thermophilic tests (Figure 16B) differed more between each other; the T8 test was the only condition showing values below 0.6 COD/COD, while T12 and T8-P have values similar to the mesophilic tests, around 0.7 COD/COD. The worst results were obtained by the T8, so the mesophilic condition seems again preferable to the thermophilic one. The similar ratios obtained by all the tests lead to think that the conversion efficiency is

not affected by the concentration of soluble organic matter, and around 30% of the sCOD always remained unconverted to SCFAs in all different fermentative conditions tested. These results are in line with the values found by Valentino et al. (2019) (0.72 mesophilic, 0.59 thermophilic) on OFMSW, and by Presti et al. (2021) (0.69 g COD_{SCFA}/L) on sewage sludge, while other literature report values of 0.81 ± 0.3 (Micolucci et al. 2020) using food waste. Lower values, between 0.4-0.6, were obtained by Morgan-Sagastume et al. (2010), where the Cambi pretreatment was performed on WAS and sCOD values were between 31-40 g O_2/L; in fact, compared to the T8-P, the sCOD is higher, but the lower conversion efficiency led to similar final concentration of SCFAs (around 20 g COD_{SCFA}/L).

4.5 Fermentation yield

The yields showed in Figure 17 were calculated as the ratio between the maximum SCFAs produced and initial VS concentration (g COD_{SCFA}/g $VS_{(0)}$). Comparing the tests with the same TS/pretreatment, but different temperature, the results show similar values. This suggest that the temperature did not have noticeable effect on fermentation yields. The higher amounts of VS in each test slightly increased the yields, shifting the average values from 0.16 (M8) to 0.18 (M12) in mesophilic conditions, and from 0.12 (T8) to 0.16 (T12) in thermophilic conditions. The pretreatment greatly improved the yields in both the mesophilic (M8-P, 0.30 g COD_{SCFA}/gVS) and thermophilic (T8-P, 0.31 g COD_{SCFA}/g VS) conditions, doubling the values. Consequently, it can be observed that the MW-H_2O_2 pretreatment led to an enhanced solubilization of the organic compounds into nutrients available for microorganisms. Moreover, the dosage of 0.2 g H_2O_2/g TS generates low
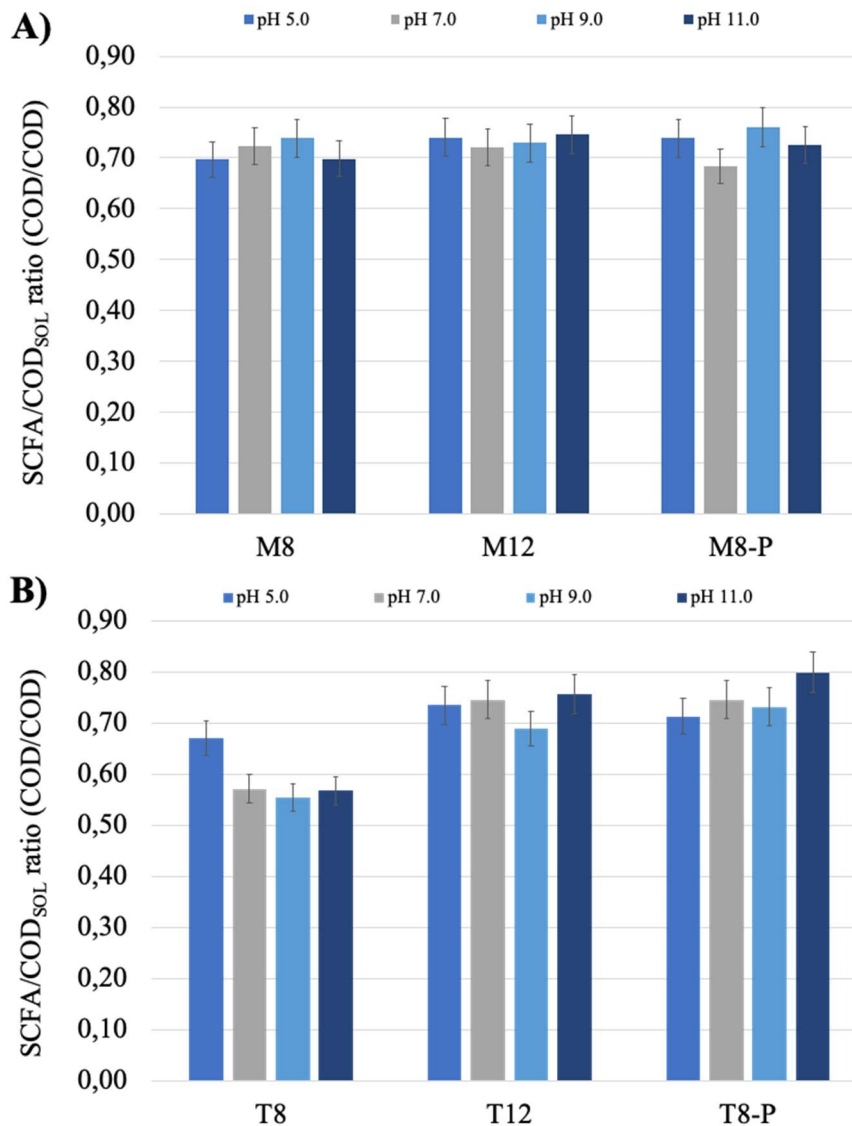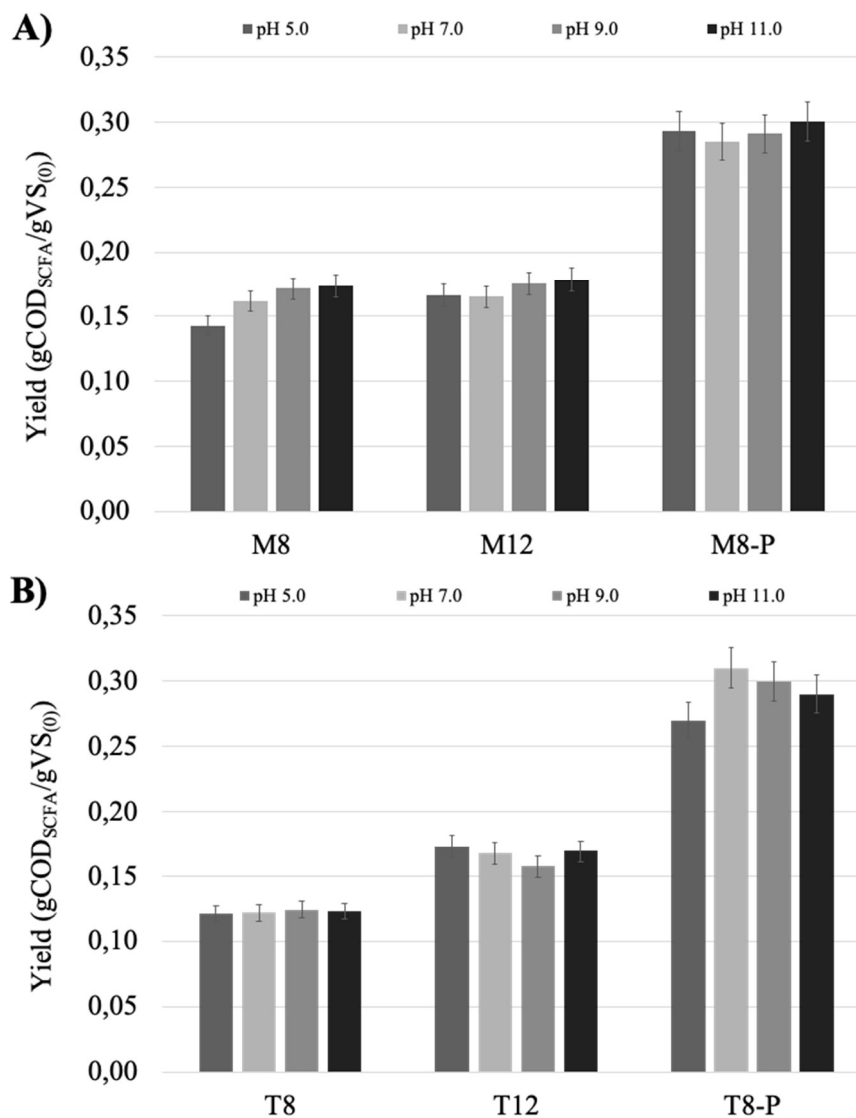
Figure 17 Yield values (gSCFA/gVS$_{(0)}$) of the mesophilic batch tests M8, M12, and M8-P under initial pH 5, 7, 9, and 11 (A); thermophilic batch tests T8, T12, and T8-P under initial pH 5, 7, 9 and 11 (B).

residual H_2O_2 and did not have an inhibitory effect on the microbial growth. Given the similar results obtained at 40°C and 55°C, the mesophilic condition appears preferable. Valentino et al. (2019) reported yields around 0.41-0.44 g COD$_{SCFA}$/g VS, independently from the chosen process temperature (37 and 55°C), using a mixture of OFMSW and WAS. The thermal pretreatment on WAS performed by Lorini et al. (2022) led to a significant COD and VS solubilization, obtaining a yield of 0.38 ± 0.04 g COD$_{SCFA}$/g VS.

Morgan-Sagastume et al. (2015) fermented thickened WAS at different temperatures (35-42-55°C), obtaining the best yields: 0.33 ± 0.03 and 0.27 ± 0.02 g COD$_{SCFA}$/g VS at 42°C and 55°C respectively. The yields obtained from tannery sludge reported in this study are comparable to those obtained from WAS only when the combined MW-H$_2$O$_2$ pretreatment was performed. When the mixture is used, the yields values are higher due to the higher fermentability of the OFMSW (Valentino et al. 2019).

4.6 SCFAs composition

As no influence of the initial pH was observed between the different tests, the graph reported in Figure 18-19 are the mean values of the four bottles corresponding to the four initial pH conditions. The abundances of each SCFA are reported as the ratio (%, gCOD$_{ACID}$/gCOD$_{SCFA}$) between the specific acid concentration and the total SCFAs concentration. In both the temperature conditions, the most abundant SCFA is acetic acid, followed by butyric acid (20-22% in mesophilic, 24-26%% in thermophilic conditions). Isovaleric acid is third in abundance at 40°C (11-16%), while at 55°C the fraction of propionic acid is slightly higher (15-16%). The mesophilic series (Figure 18) shows similar values between M8 and M12 tests, with a slight increase from 37% to 41% of the acetic fraction. The remaining fraction is composed by propionic (10-13%), isobutyric (6-8%), and valeric (1-5%) acids. The M8-P have the highest fraction of acetic acid among all tests (52%), while valeric acid almost disappear. The thermophilic series (Figure 19) also shows a slight increase of acetic acid from T8 (37%) to T12 (39%) and the pretreated T8-P (40%). Isovaleric (13-15%) and isobutyric (7-8%) acids make up the remaining composition. The valeric acid completely disappeared.
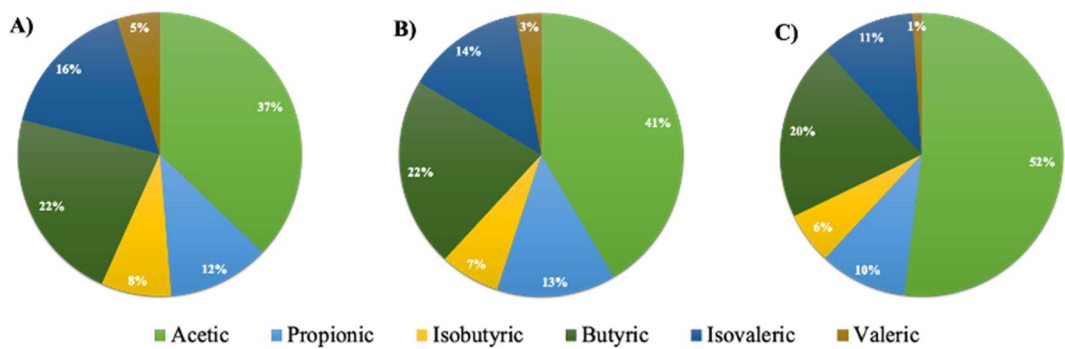
Figure 18 Composition in terms of %COD of the SCFAs obtained from the mesophilic batch tests M8 (A), M12 (B), and M8-P (C) as the average of all the initial pH conditions.
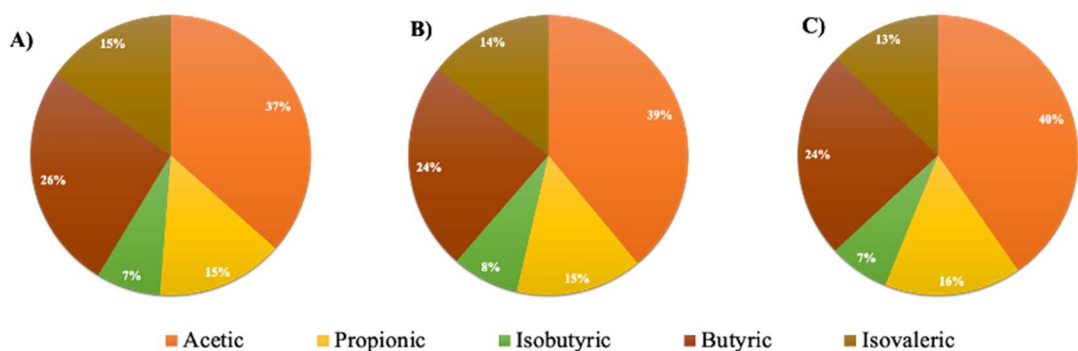
Figure 19 Composition in terms of %COD of the SCFAs obtained from the mesophilic batch tests M8 (A), M12 (B), and M8-P (C) as the average of all the initial pH conditions.

Compared to the acidogenic fermentation of OFMSW-WAS mixture (Valentino et al. 2019), the two dominant SCFAs were the same of this study, but at different proportions, as butyric acid was 41-42% (COD basis), and acetic acid was 22-23%, (COD basis), followed by propionic acid (11-10%), and a non-negligible amount valeric acid (12-11%), which is either absent, or very low in our results. The predominance of the butyric acid over the acetic acid is probably due to the use of OFMSW (Girotto et al. 2017). In Lorini et al. (2022), the most abundantly observed acids were acetic, butyric, and propionic for the thermophilic WAS fermentation (55-22-18% respectively) and the thermally pretreated WAS fermentation (58-19-15% respectively). Similar composition, but with less predominance of these three acids, was obtained by Morgan-Sagastume et al. (2015)

using thickened WAS as substrate for acidogenic fermentation (results obtained at 42 and 55°C, COD basis), with 28-38% of acetic, 15-26% butyric, 13-23% propionic, 12-18% isovaleric, and 4-11% valeric acids. These results are comparable to the series performed in this study, confirming the similarity between WAS and tannery sludge on the final SCFAs composition via acidogenic fermentation.

4.7 Perspective of tannery sludge utilization in a new biorefinery value-chain

Due to the growing concern on greenhouse gasses emissions, the transition towards a Circular Economy approach is now required in most of the productive sectors. In this context, biorefinery represents an innovative approach in the environmental management, where products at the end of their service life or waste materials, such as the organic waste, are seen as valuable resources for the production of high added value bio-products or biofuels (Nghiem et al. 2017). The potential of microbial fermentation and recovery of SCFAs from tannery sludge has never been investigated in detail, so this study aim to furnish a basis on which other scale-up experiments can rely on. The results obtained in this work are summarized in Tables 3-4. The final concentration of SCFAs reached is an important parameter, since downstream processing is also a challenge, and greater focus is needed in this area to recover SCFAs with a higher productivity and purity (Mkhize et al. 2014). The amounts of SCFAs potentially extractable from the liquid phase are significant, with no risk related to the Cr(VI) release (the analysis reported a concentration below the LOQ of 0.03 mg/L), even when compared to other organic wastes. Nevertheless, the chromium content remains an issue and its final dispose on the unused fluxes should be considered. The interest in biorefinery emerges in an increasing in the research of new and efficacy pretreatment methods, such as microwave (Strazzera et al.

2018), where an environmental and economic evaluation is recommended because of the required energy and reagents demand, which is not always recovered through an adequate increase of SCFAs yield (Bolzonella et al. 2018). Given the results obtained in this work, the pretreatment seems a more relevant aspect compared to the operative temperature, so more effort should be put on the study of technical and economic feasibility of the initial treatment of the sludge in a scale-up approach.

Table 3 Summary of the main results (average data) obtained in the mesophilic (M8, M12, M8-P) anaerobic fermentation of tannery sludge.

Parameter	Unit	Series M8				Series M12				Series M8-P			
		pH 5	pH 7	pH 9	pH 11	pH 5	pH 7	pH 9	pH 11	pH 5	pH 7	pH 9	pH 11
SCFAs	g COD/L	9.49	9.35	9.59	10.3	14.7	14.6	15.5	15.8	17.3	16.8	17.1	17.7
SCFAs/COD_{SOL}	COD/COD	0.72	0.72	0.74	0.70	0.74	0.72	0.73	0.75	0.75	0.70	0.76	0.73
Y_F	$gCOD_{SCFA}/gVS_0$	0.16	0.16	0.17	0.17	0.17	0.17	0.18	0.18	0.29	0.28	0.29	0.30
Acetic acid	g COD/L	4.73	4.73	4.52	4.23	6.22	6.13	6.78	6.05	9.06	7.95	9.86	8.51
Propionic acid	g COD/L	1.33	1.25	1.07	1.21	1.79	2.08	2.18	2.18	1.88	2.00	1.28	1.81
Isobutyric acid	g COD/L	0.57	0.88	0.82	0.71	0.99	0.99	1.04	1.14	0.93	1.00	0.99	1.09
Butyric acid	g COD/L	1.71	1.38	1.87	2.23	3.59	2.93	3.23	3.40	3.36	3.39	3.09	4.30
Isovaleric acid	g COD/L	0.50	0.89	1.00	1.48	1.94	2.02	2.11	2.15	1.83	1.91	1.81	1.81
Valeric acid	g COD/L	0.65	0.21	0.33	0.35	0.21	0.5	0.18	0.89	0.25	0.17	0.17	0.21
COD_{SOL}	g COD/L	13.1	13.0	12.5	14.7	19.9	20.3	21.3	21.2	23.0	23.8	22.6	24.4
$N-NH_4^+$	mg/L	720	645	707	680	1132	1204	1167	1221	1620	1610	1638	1550
$P-PO_4^{3-}$	mg/L	0.93	1.17	0.99	1.56	1.77	2.34	1.88	2.02	2.62	2.55	2.16	2.26
Chromium VI	mg/L	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03

Table 4 Summary of the main results (average data) obtained in the thermophilic (T8, T12, T8-P) anaerobic fermentation of tannery sludge.

Parameter	Unit	Series T8				Series T12				Series T8-P			
		pH 5	pH 7	pH 9	pH 11	pH 5	pH 7	pH 9	pH 11	pH 5	pH 7	pH 9	pH 11
SCFAs	g COD/L	7.15	7.19	7.34	7.26	15.35	14.84	13.92	14.97	23.76	26.20	26.09	25.74
SCFAs/COD_{SOL}	COD/COD	0.58	0.57	0.55	0.57	0.64	0.65	0.60	0.62	0.71	0.74	0.73	0.75
Y_F	$gCOD_{SCFA}/gVS_0$	0.12	0.12	0.12	0.12	0.17	0.17	0.16	0.17	0.28	0.31	0.30	0.29
Acetic acid	g COD/L	2.91	2.80	2.41	2.45	5.90	5.89	5.48	5.74	12.25	10.34	10.86	10.46
Propionic acid	g COD/L	1.06	1.09	1.06	1.04	2.02	2.15	2.46	2.02	3.38	4.30	4.01	3.45
Isobutyric acid	g COD/L	0.49	0.52	0.60	0.55	1.22	1.14	1.07	1.15	1.34	1.98	2.05	1.59
Butyric acid	g COD/L	1.73	1.71	2.01	2.10	3.90	3.48	2.92	3.96	4.23	6.34	5.65	6.97
Isovaleric acid	g COD/L	0.95	1.08	1.26	1.13	2.31	2.19	2.00	2.10	2.57	3.26	3.51	3.27
Valeric acid	g COD/L	-	-	-	-	-	-	-	-	-	-	-	-
COD_{SOL}	g COD/L	12.4	12.6	13.2	12.8	23.8	22.9	23.2	24.0	33.3	35.2	35.7	34.2
$N-NH_4^+$	mg/L	880	725	810	741	1268	1284	1218	1131	1432	1569	1665	1679
$P-PO_4^{3-}$	mg/L	2.2	2.2	2.6	22	2.8	2.8	2.8	2.5	2.6	2.7	2.9	2.8
Chromium VI	mg/L	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03

4 Conclusion

The aim of this study was to evaluate whether tannery sludge can be used as renewable carbon source for the production of intermediate compounds (SCFAs) in a process of acidogenic fermentation and assessing the most appropriate operative conditions in terms of temperature, pH, solids content, and oxidizing/heating pretreatment. The main parameters to evaluate the process performances were the final concentration of SCFAs, SCFA/sCOD ratio, yield, and composition. In the batch tests performed, the best results were obtained under thermophilic conditions (55°C) combined with the MW-H_2O_2 pretreatment (T8-P test), with a concentration of organic acids between 23.7-26.2 g COD_{SCFA}/L, a conversion efficiency of more than 0.7 (g COD_{SCFA}/g sCOD) and a yield of 0.31 g COD_{SCFA}/g VS. Between the two different temperatures investigated no substantial differences in the yields and the SCFA/sCOD ratios were observed, while the final concentrations of SCFAs is lower in the mesophilic condition only when the pretreatment is performed; however, the time required by the T8-P to reach the plateau was longer than 24 days, making it difficult to manage in a continuous process. In the mesophilic series, the best results were given by the pretreated tests (M8-P), with a final concentration slightly higher (16.8-17.7 g COD_{SCFA}/L) compared to the M12 (14.6-15.8 g COD_{SCFA}/L), and a SCFA/sCOD ratio (0.7 COD/COD) similar for all the tests of the series. The best benefit of the MW-H_2O_2 pretreatment is shown by the yield, 0.30 g COD_{SCFA}/g VS, double compared to the M12 and M8. Overall, the mesophilic condition had better results and, being also less energy intensive and easier to perform in large scale, it appears to be a more appealing alternative compared to the thermophilic one. These results are comparable with those found in the literature on the acidogenic fermentation of WAS, even when thermal pretreatment was performed, while better results were found

by other authors when a mixture of WAS with a more fermentable organic waste was used (Valentino et al. 2019). An interesting characteristic observed in this study is the characteristic of the tannery sludge to act as a buffer and maintain a neutral pH (around 7), due to the presence of lime deriving from the tanning process and treatment of tannery wastewater. Usually, when the pH values drop to less than 5.25 the activity of the microorganism is inhibited, and during the process the accumulation of SCFAs tends to decrease the pH. This normally require the addition of chemicals to maintain an appropriate pH during the process, which is not required with this particular sludge.

As previously mentioned, the tannery industry has a process variability due to the different raw materials and chemicals used during the year, so further work should be done to explore the different characteristics of resulting wastes and optimize the related valorisation process. These results represent a first step in the understanding of this resource and further work is needed to assess the best working conditions to fully take advantage of this waste. Further research should be performed on the economic and technical feasibility of what assessed in this work, and on the integration of this process in the already existing tannery WWTPs. The valorization of this renewable carbon source, through the recovery of bio-materials precursors, would develop an integrated process of Circular Economy according to the requirements of the European Green Deal, and address environmentally friendly practices in one of the most polluting sectors worldwide.

5 REFERENCES

Akyol, Ç., Demirel, B., & Onay, T. T. (2015). Recovery of methane from tannery sludge: the effect of inoculum to substrate ratio and solids content. *Journal of Material Cycles and Waste Management*, *17*(4), 808–815. https://doi.org/10.1007/s10163-014-0306-2.

Alibardi, Luca, and Raffaello Cossu. 2016. "Pre-Treatment of Tannery Sludge for Sustainable Landfilling." *Waste Management* 52 (June): 202–11. https://doi.org/10.1016/j.wasman.2016.04.008.

Ambrose, H. W., Chin, C. T. L., Hong, E., Philip, L., Suraishkumar, G. K., Sen, T. K., & Khiadani, M. (2020). Effect of hybrid (microwave-H2O2) feed sludge pretreatment on single and two-stage anaerobic digestion efficiency of real mixed sewage sludge. *Process Safety and Environmental Protection*, *136*, 194–202. https://doi.org/10.1016/j.psep.2020.01.032.

Andersen, R. L., Jensen, K. M., & Mikkelsen, M. J. (2015). Continuous ethanol fermentation of pretreated lignocellulosic biomasses, waste biomasses, molasses and syrup using the anaerobic, thermophilic bacterium Thermoanaerobacter italicus pentocrobe 411. *PLoS ONE*, *10*(8). https://doi.org/10.1371/journal.pone.0136060.

Atasoy, M., Owusu-Agyeman, I., Plaza, E., & Cetecioglu, Z. (2018, November 1). Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges. *Bioresource Technology*. Elsevier Ltd. https://doi.org/10.1016/j.biortech.2018.07.042.

Atasoy, M., Eyice, O., Schnürer, A., & Cetecioglu, Z. (2019). Volatile fatty acids production via mixed culture fermentation: Revealing the link between pH, inoculum type and bacterial composition. *Bioresource Technology*, *292*. https://doi.org/10.1016/j.biortech.2019.121889.

Banu, J. R., Kaliappan, S., & Yeom, I. T. (2007). Treatment of domestic wastewater using upflow anaerobic sludge blanket reactor. *International Journal of Environmental Science and Technology*, *4*(3), 363–370. https://doi.org/10.1007/BF03326295.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V., Pavlostathis, S. G., Rozzi, A., … Vavilin, V. A. (2002). The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Science and Technology : A Journal of the International Association on Water Pollution Research*, *45*(10), 65–73. https://doi.org/10.2166/wst.2002.0292.

Battista, F., Gomez Almendros, M., Rousset, R., Boivineau, S., & Bouillon, P. A. (2018). Enzymatic hydrolysis at high dry matter content: The influence of the substrates' physical properties and of loading strategies on mixing and energetic consumption. *Bioresource Technology*, *250*, 191–196. https://doi.org/10.1016/j.biortech.2017.11.049.

Baumann, I., & Westermann, P. (2016). Microbial Production of Short Chain Fatty Acids from Lignocellulosic Biomass: Current Processes and Market. *BioMed Research International*. Hindawi Limited. https://doi.org/10.1155/2016/8469357.

Bautista, M. E., Pérez, L., García, M. T., Cuadros, S., & Marsal, A. (2015). Valorization of tannery wastes: Lipoamino acid surfactant mixtures from the protein fraction of process wastewater. *Chemical Engineering Journal, 262*, 399–408. http://doi.org/10.1016/j.cej.2014.10.004.

Bengtsson, S., Hallquist, J., Werker, A., & Welander, T. (2008). Acidogenic fermentation of industrial wastewaters: Effects of chemostat retention time and pH on volatile fatty acids production. *Biochemical Engineering Journal*, *40*(3), 492–499. https://doi.org/10.1016/j.bej.2008.02.004.

Bhatia, S. K., & Yang, Y. H. (2017, June 1). Microbial production of volatile fatty acids: current status and future perspectives. *Reviews in Environmental Science and Biotechnology*. Springer Netherlands. https://doi.org/10.1007/s11157-017-9431-4.

Böhler, M., & Siegrist, H. (2004). Partial ozonation of activated sludge to reduce excess sludge, improve denitrification and control scumming and bulking. *Water Science and Technology*, *49*(10), 41–49. https://doi.org/10.2166/wst.2004.0604.

Bolzonella, D., Battista, F., Cavinato, C., Gottardo, M., Micolucci, F., Lyberatos, G., & Pavan, P. (2018, June 1). Recent developments in biohythane production from household food wastes: A review. *Bioresource Technology*. Elsevier Ltd. https://doi.org/10.1016/j.biortech.2018.02.092.

Cesaro, A., & Belgiorno, V. (2020). Ozone pretreatment for the anaerobic digestion of organic solid waste. *Detritus*, *12*, 51–56. https://doi.org/10.31025/2611-4135/2020.13990.

Cheah, Y. K., Dosta, J., & Mata-Álvarez, J. (2019). Enhancement of volatile fatty acids production from food waste by mature compost addition. *Molecules*, *24*(16). https://doi.org/10.3390/molecules24162986.

Chiavola, A., Salvati, C., Bongirolami, S., Di Marcantonio, C., & Boni Maria, R. (2021). Techno-economic evaluation of ozone-oxidation for sludge reduction at the full-scale. Comparison between the application to the return activated sludge (RAS) and the sludge digestion unit. *Journal of Water Process Engineering*, *42*. https://doi.org/10.1016/j.jwpe.2021.102114.

Daryapurkar, R. A., Nandy, T., Kaul, S. N., Deshpande, C. V., & Szpyrkowicz, L. (2001). Evaluation of kinetic constants for anaerobic fixed film fixed bed reactors treating tannery wastewater. *International Journal of Environmental Studies*, *58*(6), 835–860. https://doi.org/10.1080/00207230108711371.

Ec.europa.eu;(https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_it).

Eskicioglu, C., Prorot, A., Marin, J., Droste, R. L., & Kennedy, K. J. (2008). Synergetic pretreatment of sewage sludge by microwave irradiation in presence of H2O2 for enhanced anaerobic digestion. *Water Research*, *42*(18), 4674–4682. https://doi.org/10.1016/j.watres.2008.08.010.

Fonseca, Y. A. D., Silva, N. C. S., De Camargos, A. B., Silva, S. D. Q., Wandurraga, H. J. L., Gurgel, L. V. A., & Baêta, B. E. L. (2021). Influence of hydrothermal pretreatment conditions, typology of anaerobic digestion system, and microbial profile in the production of volatile fatty acids from olive mill solid waste. *Journal of Environmental Chemical Engineering*, *9*(2). https://doi.org/10.1016/j.jece.2021.105055.

Fei, Q., Fu, R., Shang, L., Brigham, C. J., & Chang, H. N. (2015). Lipid production by microalgae Chlorella protothecoides with volatile fatty acids (VFAs) as carbon sources in heterotrophic cultivation and its economic assessment. *Bioprocess and Biosystems Engineering*, *38*(4), 691–700. https://doi.org/10.1007/s00449-014-1308-0.

Feng, X. H., Chen, F., Xu, H., Wu, B., Yao, J., Ying, H. J., & Ouyang, P. K. (2010). Propionic acid fermentation by Propionibacterium freudenreichii CCTCC M207015 in a multi-point fibrous-bed bioreactor. *Bioprocess and Biosystems Engineering*, *33*(9), 1077–1085. https://doi.org/10.1007/s00449-010-0433-7.

Garcia-Aguirre, J., Aymerich, E., González-Mtnez. de Goñi, J., & Esteban-Gutiérrez, M. (2017). Selective VFA production potential from organic waste streams: Assessing temperature and pH influence. *Bioresource Technology*, *244*, 1081–1088. https://doi.org/10.1016/j.biortech.2017.07.187.

Ghysels, S., Acosta, N., Estrada, A., Pala, M., De Vrieze, J., Ronsse, F., & Rabaey, K. (2020). Integrating anaerobic digestion and slow pyrolysis improves the product portfolio of a cocoa waste biorefinery. *Sustainable Energy and Fuels*, *4*(7), 3712–3725. https://doi.org/10.1039/d0se00689k.

Giaccherini, F. (2017). Modelling tannery wastewater treatment to evaluate alternative bioprocesses configurations (Doctoral dissertation, Technische Universität Braunschweig).

Girotto, F., Lavagnolo, M. C., Pivato, A., & Cossu, R. (2017). Acidogenic fermentation of the organic fraction of municipal solid waste and cheese whey for bio-plastic precursors recovery – Effects of process conditions during batch tests. *Waste Management*, *70*, 71–80. https://doi.org/10.1016/j.wasman.2017.09.015.

Hansen, C.L., Cheong, D.Y. (2013). Agricultural Waste Management in Food Processing. Handbook of Farm, Dairy, and Food Machinery Engineering; Academic Press: Cambridge, MA, USA; ISBN 978-0-12-385881-8.

Hayyan, M., Hashim, M. A., & Alnashef, I. M. (2016, March 9). Superoxide Ion: Generation and Chemical Implications. *Chemical Reviews*. American Chemical Society. https://doi.org/10.1021/acs.chemrev.5b00407.

He, M., Sun, Y., Zou, D., Yuan, H., Zhu, B., Li, X., & Pang, Y. (2012). Influence of Temperature on Hydrolysis Acidification of Food Waste. *Procedia Environmental Sciences*, *16*, 85–94. https://doi.org/10.1016/j.proenv.2012.10.012.

Karthikeyan, O. P., & Visvanathan, C. (2013, September). Bio-energy recovery from high-solid organic substrates by dry anaerobic bio-conversion processes: A review. *Reviews in Environmental Science and Biotechnology*. https://doi.org/10.1007/s11157-012-9304-9.

Kim, M., Gomec, C. Y., Ahn, Y., & Speece, R. E. (2003). Hydrolysis and acidogenesis of particulate organic material in mesophilic and thermophilic anaerobic digestion. *Environmental Technology (United Kingdom)*, *24*(9), 1183–1190. https://doi.org/10.1080/09593330309385659.

Kim, J. K., Oh, B. R., Chun, Y. N., & Kim, S. W. (2006). Effects of temperature and hydraulic retention time on anaerobic digestion of food waste. *Journal of Bioscience and Bioengineering*, *102*(4), 328–332. https://doi.org/10.1263/jbb.102.328.

Kuglarz, M., Karakashev, D., & Angelidaki, I. (2013). Microwave and thermal pretreatment as methods for increasing the biogas potential of secondary sludge from municipal wastewater treatment plants. *Bioresource Technology*, *134*, 290–297. https://doi.org/10.1016/j.biortech.2013.02.001.

Lee, W. S., Chua, A. S. M., Yeoh, H. K., & Ngoh, G. C. (2014, January 1). A review of the production and applications of waste-derived volatile fatty acids. *Chemical Engineering Journal*. https://doi.org/10.1016/j.cej.2013.09.002.

Lefebvre, O., Vasudevan, N., Torrijos, M., Thanasekaran, K., & Moletta, R. (2006). Anaerobic digestion of tannery soak liquor with an aerobic post-treatment. *Water Research*, *40*(7), 1492–1500. https://doi.org/10.1016/j.watres.2006.02.004.

Li, Y., Park, S. Y., & Zhu, J. (2011). Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Reviews*. Elsevier Ltd. https://doi.org/10.1016/j.rser.2010.07.042.

Lim, S. J., Kim, B. J., Jeong, C. M., Choi, J. dal rae, Ahn, Y. H., & Chang, H. N. (2008). Anaerobic organic acid production of food waste in once-a-day feeding and drawing-off bioreactor. *Bioresource Technology*, *99*(16), 7866–7874. https://doi.org/10.1016/j.biortech.2007.06.028.

Liu, H., Wang, J., Liu, X., Fu, B., Chen, J., & Yu, H. Q. (2012). Acidogenic fermentation of proteinaceous sewage sludge: Effect of pH. *Water Research*, *46*(3), 799–807. https://doi.org/10.1016/j.watres.2011.11.047.

Liu, J., Jia, R., Wang, Y., Wei, Y., Zhang, J., Wang, R., & Cai, X. (2017). Does residual H2O2 result in inhibitory effect on enhanced anaerobic digestion of sludge pretreated by microwave-H2O2 pretreatment process? *Environmental Science and Pollution Research*, *24*(10), 9016–9025. https://doi.org/10.1007/s11356-015-5704-z.

Liu, J., Yang, M., Zhang, J., Zheng, J., Xu, H., Wang, Y., & Wei, Y. (2018a). A comprehensive insight into the effects of microwave-H2O2 pretreatment on concentrated sewage sludge anaerobic digestion based on semi-continuous operation. *Bioresource Technology*, *256*, 118–127. https://doi.org/10.1016/j.biortech.2018.01.126.

Liu, N., Jiang, J., Yan, F., Xu, Y., Yang, M., Gao, Y., … Zou, Q. (2018b). Optimization of simultaneous production of volatile fatty acids and bio-hydrogen from food waste using response surface methodology. *RSC Advances*, *8*(19), 10457–10464. https://doi.org/10.1039/c7ra13268a.

Liu, H., Wang, Y., Zhang, H., Huang, G., Yang, Q., & Wang, Y. (2019). Synchronous detoxification and reduction treatment of tannery sludge using Cr (VI) resistant bacterial strains. *Science of the Total Environment*, *687*, 34–40. https://doi.org/10.1016/j.scitotenv.2019.06.093.

Lorini, L., di Re, F., Majone, M., & Valentino, F. (2020). High rate selection of PHA accumulating mixed cultures in sequencing batch reactors with uncoupled carbon and nitrogen feeding. *New Biotechnology*, *56*, 140–148. https://doi.org/10.1016/j.nbt.2020.01.006.

Lorini, L., Munarin, G., Salvatori, G., Alfano, S., Pavan, P., Majone, M., & Valentino, F. (2022). Sewage sludge as carbon source for polyhydroxyalkanoates: a holistic approach at pilot scale level. *Journal of Cleaner Production*, *354*, 131728. https://doi.org/10.1016/j.jclepro.2022.131728.

Mannucci, A., Munz, G., Mori, G., & Lubello, C. (2010). Anaerobic treatment of vegetable tannery wastewaters: A review. DES, 264(1-2), 1–8. http://doi.org/10.1016/j.desal.2010.07.021.

Meegoda, J. N., Li, B., Patel, K., & Wang, L. B. (2018, October 11). A review of the processes, parameters, and optimization of anaerobic digestion. *International Journal of Environmental Research and Public Health*. MDPI AG. https://doi.org/10.3390/ijerph15102224.

Micolucci, F., Gottardo, M., Bolzonella, D., Pavan, P., Majone, M., & Valentino, F. (2020). Pilot-scale multi-purposes approach for volatile fatty acid production, hydrogen and methane from an automatic controlled anaerobic process. *Journal of Cleaner Production*, *277*. https://doi.org/10.1016/j.jclepro.2020.124297.

Mkhize, N. T., Msagati, T. A. M., Mamba, B. B., & Momba, M. (2014). Determination of volatile fatty acids in wastewater by solvent extraction and gas chromatography. *Physics and Chemistry of the Earth*, *67–69*, 86–92. https://doi.org/10.1016/j.pce.2013.10.008.

Morgan-Sagastume, F., Karlsson, A., Johansson, P., Pratt, S., Boon, N., Lant, P., & Werker, A. (2010). Production of polyhydroxyalkanoates in open, mixed cultures from a waste sludge stream containing high levels of soluble organics, nitrogen and

phosphorus. *Water Research*, *44*(18), 5196–5211.
https://doi.org/10.1016/j.watres.2010.06.043.

Motte, J. C., Trably, E., Escudié, R., Hamelin, J., Steyer, J. P., Bernet, N., … Dumas, C. (2013). Total solids content: A key parameter of metabolic pathways in dry anaerobic digestion. *Biotechnology for Biofuels*, *6*(1). https://doi.org/10.1186/1754-6834-6-164.

Mpofu, A. B., Oyekola, O. O., & Welz, P. J. (2021, May 10). Anaerobic treatment of tannery wastewater in the context of a circular bioeconomy for developing countries. Journal of Cleaner Production. Elsevier Ltd. https://doi.org/10.1016/j.jclepro.2021.126490.

Mwinyihija, M. (2010). *Ecotoxicological diagnosis in the tanning industry*. *Ecotoxicological Diagnosis in the Tanning Industry* (pp. 1–140). Springer New York. https://doi.org/10.1007/978-1-4419-6266-9.

Naresh Kumar, A., Sarkar, O., Chandrasekhar, K., Raj, T., Narisetty, V., Mohan, S. V., … Kim, S. H. (2022, February 1). Upgrading the value of anaerobic fermentation via renewable chemicals production: A sustainable integration for circular bioeconomy. *Science of the Total Environment*. Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2021.150312.

Nghiem, L. D., Koch, K., Bolzonella, D., & Drewes, J. E. (2017). Full scale co-digestion of wastewater sludge and food waste: Bottlenecks and possibilities. *Renewable and Sustainable Energy Reviews*. Elsevier Ltd. https://doi.org/10.1016/j.rser.2017.01.062.

Oleskowicz-Popiel, P., Kádár, Z., Heiske, S., Klein-Marcuschamer, D., Simmons, B. A., Blanch, H. W., & Schmidt, J. E. (2012). Co-production of ethanol, biogas, protein fodder and natural fertilizer in organic farming - Evaluation of a concept for a farm-scale biorefinery. *Bioresource Technology*, *104*, 440–446. https://doi.org/10.1016/j.biortech.2011.11.060.

Patel, A., Sarkar, O., Rova, U., Christakopoulos, P., & Matsakas, L. (2021, February 1). Valorization of volatile fatty acids derived from low-cost organic waste for lipogenesis in oleaginous microorganisms-A review. *Bioresource Technology*. Elsevier Ltd. https://doi.org/10.1016/j.biortech.2020.124457.

Pommier, S., Chenu, D., Quintard, M., & Lefebvre, X. (2007). A logistic model for the prediction of the influence of water on the solid waste methanization in landfills. *Biotechnology and Bioengineering*, *97*(3), 473–482. https://doi.org/10.1002/bit.21241.

Presti, D., Cosenza, A., Capri, F. C., Gallo, G., Alduina, R., & Mannina, G. (2021). Influence of volatile solids and pH for the production of volatile fatty acids: Batch fermentation tests using sewage sludge. *Bioresource Technology*, *342*. https://doi.org/10.1016/j.biortech.2021.125853.

Rani, P., Kumar, A., & Arya, R. C. (2017). Stabilization of tannery sludge amended soil using Ricinus communis, Brassica juncea and Nerium oleander. *Journal of Soils and Sediments*, *17*(5), 1449–1458. https://doi.org/10.1007/s11368-016-1466-6.

Rebecchi, S., Pinelli, D., Bertin, L., Zama, F., Fava, F., & Frascari, D. (2016). Volatile fatty acids recovery from the effluent of an acidogenic digestion process fed with grape pomace by adsorption on ion exchange resins. *Chemical Engineering Journal*, *306*, 629–639. https://doi.org/10.1016/j.cej.2016.07.101.

Santos, J. A., Nunes, L. A. P. L., Melo, W. J., & Araújo, A. S. F. (2011). Tannery sludge compost amendment rates on soil microbial biomass of two different soils. *European Journal of Soil Biology*, *47*(2), 146–151. https://doi.org/10.1016/j.ejsobi.2011.01.002.

Song, Z., Williams, C. J., & Edyvean, R. G. J. (2000). Sedimentation of tannery wastewater. *Water Research*, *34*(7), 2171–2176. https://doi.org/10.1016/S0043-1354(99)00358-9.

Soomro, A. F., Abbasi, I. A., Ni, Z., Ying, L., & Liu, J. (2020). Influence of temperature on enhancement of volatile fatty acids fermentation from organic fraction of municipal solid waste: Synergism between food and paper components. *Bioresource Technology*, *304*. https://doi.org/10.1016/j.biortech.2020.122980.

Strazzera, G., Battista, F., Garcia, N. H., Frison, N., & Bolzonella, D. (2018). Volatile fatty acids production from food wastes for biorefinery platforms: A review. *Journal of Environmental Management*, *226*, 278–288. https://doi.org/10.1016/j.jenvman.2018.08.039.

Swartz, C. D., Jackson-Moss, C., Rowswell, R. A., Mpofu, A. B., & Welz, P. J. (2017). Water and wastewater management in the tanning and leather finishing industry: Natsurv 10. Water Research Commission, Republic of South Africa.

Tan, G. Y. A., Chen, C. L., Li, L., Ge, L., Wang, L., Razaad, I. M. N., … Wang, J. Y. (2014). Start a research on biopolymer polyhydroxyalkanoate (PHA): A review. *Polymers*. Molecular Diversity Preservation International. https://doi.org/10.3390/polym6030706.

Tyagi, V. K., & Lo, S. L. (2013). Microwave irradiation: A sustainable way for sludge treatment and resource recovery. *Renewable and Sustainable Energy Reviews*. https://doi.org/10.1016/j.rser.2012.10.032.

Tuncay, S., Akcakaya, M., & Icgen, B. (2022). Ozonation of sewage sludge prior to anaerobic digestion led to Methanosaeta dominated biomethanation. *Fuel*, *313*. https://doi.org/10.1016/j.fuel.2021.122690.

UNIC Italian Tanneries (2010). Annual Report – Italian National Union of Tanning Industry – Rapporto Socio-Ambientale 2010 – Document in Italian. <www.unic.it>.

UNIC Italian Tanneries (2020). "Business Overview on Italian Tanning Industry."

Valentino, F., Moretto, G., Gottardo, M., Pavan, P., Bolzonella, D., & Majone, M. (2019). Novel routes for urban bio-waste management: A combined acidic fermentation and anaerobic digestion process for platform chemicals and biogas production. *Journal of Cleaner Production*, *220*, 368–375. https://doi.org/10.1016/j.jclepro.2019.02.102.

Valentino, F., Munarin, G., Biasiolo, M., Cavinato, C., Bolzonella, D., & Pavan, P. (2021). Enhancing volatile fatty acids (VFA) production from food waste in a two-phases pilot-scale anaerobic digestion process. *Journal of Environmental Chemical Engineering*, *9*(5). https://doi.org/10.1016/j.jece.2021.106062.

Vidal-Antich, C., N. Perez-Esteban, S. Astals, M. Peces, J. Mata-Alvarez, and J. Dosta. (2021). "Assessing the Potential of Waste Activated Sludge and Food Waste Co-Fermentation for Carboxylic Acids Production." *Science of the Total Environment* 757 (February). https://doi.org/10.1016/j.scitotenv.2020.143763.

Volker, A. R., Gogerty, D. S., Bartholomay, C., Hennen-Bierwagen, T., Zhu, H., & Bobik, T. A. (2014). Fermentative production of short-chain fatty acids in Escherichia coli. *Microbiology (United Kingdom)*, *160*(PART 7), 1513–1522. https://doi.org/10.1099/mic.0.078329-0.

Wang, L., Ben, W., Li, Y., Liu, C., & Qiang, Z. (2018). Behavior of tetracycline and macrolide antibiotics in activated sludge process and their subsequent removal during sludge reduction by ozone. *Chemosphere*, *206*, 184–191. https://doi.org/10.1016/j.chemosphere.2018.04.180.

Wang, D., Huang, Y., Xu, Q., Liu, X., Yang, Q., & Li, X. (2019). Free ammonia aids ultrasound pretreatment to enhance short-chain fatty acids production from waste activated sludge. *Bioresource Technology*, *275*, 163–171. https://doi.org/10.1016/j.biortech.2018.12.055.

Weiland, P. (2010). Biogas production: Current state and perspectives. *Applied Microbiology and Biotechnology*. Springer Verlag. https://doi.org/10.1007/s00253-009-2246-7.

Xiao Q, Wei Y, Wang Y, Zeng F (2012). Comparison of sludge solubilization by microwave and its combined processes. China Water & Wastewater 28:61–64.

Xu, Q., Liu, X., Wang, D., Liu, Y., Wang, Q., Ni, B. J., … Li, H. (2019). Enhanced short-chain fatty acids production from waste activated sludge by sophorolipid: Performance, mechanism, and implication. *Bioresource Technology*, *284*, 456–465. https://doi.org/10.1016/j.biortech.2019.03.121.

Zhai, S., Li, M., Xiong, Y., Wang, D., & Fu, S. (2020). Dual resource utilization for tannery sludge: Effects of sludge biochars (BCs) on volatile fatty acids (VFAs) production from sludge anaerobic digestion. *Bioresource Technology*, *316*. https://doi.org/10.1016/j.biortech.2020.123903.

Zhang, G., Yang, J., Liu, H., & Zhang, J. (2009a). Sludge ozonation: Disintegration, supernatant changes and mechanisms. *Bioresource Technology*, *100*(3), 1505–1509. https://doi.org/10.1016/j.biortech.2008.08.041.

Zhang, P., Chen, Y., & Zhou, Q. (2009b). Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: Effect of pH. *Water Research*, *43*(15), 3735–3742. https://doi.org/10.1016/j.watres.2009.05.036.

Zhao, C., & Chen, W. (2019). A review for tannery wastewater treatment: some thoughts under stricter discharge requirements. *Environmental Science and Pollution Research*, *26*(25), 26102–26111. https://doi.org/10.1007/s11356-019-05699-6.

Zhu, Y., & Yang, S. T. (2004). Effect of pH on metabolic pathway shift in fermentation of xylose by Clostridium tyrobutyricum. *Journal of Biotechnology*, *110*(2), 143–157. https://doi.org/10.1016/j.jbiotec.2004.02.006.

Zigová, J., Šturdík, E., Vandák, D., & Schlosser, Š. (1999). Butyric acid production by Clostridium butyricum with integrated extraction and pertraction. *Process Biochemistry*, *34*(8), 835–843. https://doi.org/10.1016/S0032-9592(99)00007-2.