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in **Environmental Science**

Final Thesis

Use of natural zeolites for complexed chromium removal and production of volatile fatty acids from tannery sludge

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Summary

1. Introduction

The main objectives of the European Union's 2030 strategy, in line with the Paris Agreement, focus strongly on adopting the principles of circular economy and sustainable growth (UE, 2021). In response to the increasing demand for freshwater, population growth, and the depletion of non-renewable resources, the sustainable and circular use of resources is actively promoted. Emphasis is placed on the concept of 'zero waste', considering wastewater as a renewable source (Yadav *et al.*, 2021), emphasizing its usefulness in recovering energy and high-value resources. This approach has stimulated the development and implementation of innovative waste treatment strategies, focusing on transforming waste into raw materials and promoting reuse to make systems more self-sufficient (Yadav *et al.*, 2021).

Furthermore, in the context of the UN Sustainable Development Goal (SDG) No. 6, which aims at 'Clean Water and Sanitation for All', the implementation of sustainable water treatment technologies contributes to ensuring equity and access to sanitation. The presence of heavy metals in the aquatic environment from untreated industrial discharges into water bodies and their potential impacts on living organisms emerge as serious global concerns (Hama Aziz *et al.*, 2023).

Water scarcity and security concerns pose significant challenges to global efforts to contribute to the UN SDGs while ensuring the fundamental right to clean water for vulnerable communities (Kurniawan *et al.*, 2023).

1.1 Tanneries

In the world, 8 million tons of hides from meat processing are produced annually (UNIC, 2019).

Italy is a leader in the sector with 1146 companies and 18030 workers, the volume of finished leather produced is 110 million m^3 and 9 tons of sole leather. Production value is 4.6 billion and export is 3.2 billion. These materials are mainly for footwear, leather goods, upholstery, and bodywork factories (UNIC Concerie Italiane, 2022).

Veneto, Tuscany, Campania, and Lombardy are the regions with the largest number of companies working in the tanning industry. Veneto ranks first with 444 companies with a production of 2.697 billion euros for the year 2021/2022. The Italian tanning industry accounts for 61 percent of the production volume in Europe and the world 34 percent of the export value of finished leather (UNIC Concerie Italiane, 2022).

Tanning is the process that transforms raw hides into leather. The aim is to create a product that is resistant to microbial degradation, thermal stress, and humidity using a sequence of mechanical and chemical processes (Alibardi and Cossu, 2016).

It starts with collecting raw materials, namely the skins with blood, hair, and meat still partly attached (Sivaram and Barik, 2018). The raw hides are then treated with water, surfactants, basic salts, and enzymes to restore the water content in the raw hides and remove dirt, blood, and other substances that could interfere with the tanning process. The hides are then treated with lime and sodium sulfide to remove the epidermis and hair (UNIC, 2018). Desalinization releases salts that constitute an important part of the solid waste produced by a tannery (Chowdhury *et al.*, 2013). Then, in a mechanical operation, the fat and residual tissues still adhering to the skin after skinning are removed. With descaling and degreasing, the lime is chemically removed and the residual fat on the hide is removed through surfactants. Before starting the tanning process, the leather is treated with a bath containing salt and acid. Tanning consists of treating the leather with substances that can chemically bind to the leather, making it imputrescible, most commonly this passage employs trivalent chromium sulfate (Cr(III)) as the tanning agent, due to its higher capacity to increase the mechanical strength of the final product (Alibardi and Cossu, 2016). In the final part, basification is carried out to favor the chemical tanning reaction. Then splitting, shaving, and further tanning are carried out to modify the product's characteristics. Dyeing is used to impart color to the hide (UNIC, 2018). Acids, alkalis, chromium salts, tannins, solvents, sulfides, dyes, auxiliaries, and many other compounds used in the transformation of raw hides into commercial products are not fully incorporated into the hide and remain in the effluent (Saravanabhavan *et al.*, 2004). The performance of tanning baths during chrome tanning does not exceed 50-60%, resulting in wasted raw materials and the presence of chromium in the waste (Pietrelli *et al.*, 2019).

The classification of tannery waste differs depending on the legal context of individual states and the treatment and reuse/recycling facilities available (Pietrelli *et al.*, 2019). Tannery wastes include proteins, hair, lime, salts, acids, tannins, dyes, and oils, most of which are present in the wastewater stream, which is then conveyed to centralized industrial wastewater treatment plants. After the wastewater treatment, the main byproduct left is tannery sludge, which includes activated sludge and sludge from physico-chemical treatments. The disposal of this waste represents one of the major problems in the tanning industry (Silva *et al.*, 2010; Alibardi and Cossu, 2016). Due to the solid waste's different physical, chemical, and biological properties from each process, separate treatment techniques must be used for each waste (Saira and Shanthakumar, 2023).

The environmental impact of this waste is high due to the presence of chromium and the content of solids and for this reason, it must be properly disposed of (Silva *et al.*, 2010). According to Legislative Decree 04/06, in Italy, this sludge is classified as a special non-hazardous residue, and its current destination is a second-class type B controlled landfill (Tuci *et al.*, 2022).

Currently, the management of this waste translates into its disposal in landfills, generating impacts both on an economic level for the industries involved and on the environmental front (Tuci *et al.*, 2022).

More recently, the acidogenic fermentation process has been implemented on various types of waste in numerous laboratory and pilot-scale studies and has revealed considerable potential for the recovery of valuable resources, such as short-chain fatty acids (SCFAs) (Naresh Kumar *et al.*, 2022). Some initial applications of this technology on tannery sludge are also underway, with promising results (Tuci *et al.*, 2022).

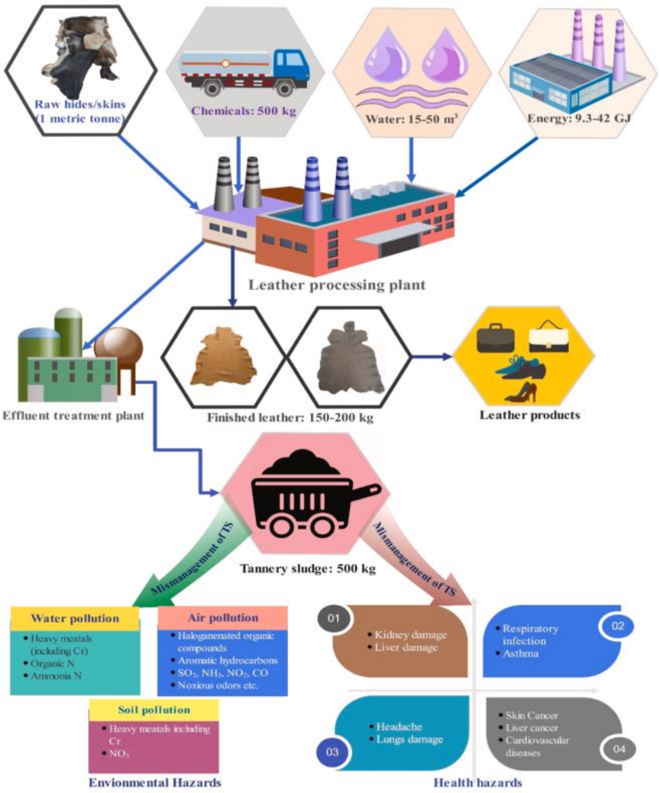


Figure 1. The complete process of leather processing along with health and environmental problems associated with Tannery sludge (Moktadir, Ren and Zhou, 2023).

1.2 Chromium

Chromium salts are used for their ease of operation, suitability for dyeing, and the excellent properties they impart to the leather at the end of the tanning process (V.J. Sundar, J. Raghava Rao, 2001). Specifically, basic chromium sulfate (BCS) provides hydrothermal stability and other unique properties to tanned leathers (Kilicarislan Ozkan, Ozgunay, and Akat, 2019).

Only a fraction of salts react with the hides, and the rest ends up in the tanning effluent, becoming waste (V.J. Sundar, J. Raghava Rao, 2001). Improper disposal of tannery wastewater on land causes the transfer of heavy metals to the soil. These metals accumulate in plant tissue and enter the food chain through biomagnification (Ahmed *et al.*, 2022).

The distribution of chromium in environmental systems is controlled by the following reactions: reduction, oxidation, precipitation, dissolution, absorption, and desorption (Salem *et al.*, 1989). The toxicity of Cr depends mainly on its chemical form (Abreu and Toffoli, 2009), as chromium can exist in different oxidation states, of which the most stable and common forms in the environment are the trivalent [Cr(III)] and hexavalent [Cr(VI)] species. These species have different chemical properties and their impact on organisms is different (Viti *et al.*, 2006).

Cr(VI) is more toxic and carcinogenic than Cr(III) and has been considered a priority pollutant (Mortazavian *et al.*, 2016). Hexavalent chromium is water-soluble, mutagenic, and toxic, and its hazard is associated with its higher mobility; on the other hand, trivalent chromium is relatively soluble and 100 times less toxic (Viti *et al.*, 2006).

In the study by Han et al., 2004, chromium (VI) was found to be more toxic than Cr(III) for many plants, such as maize, sugar beets, and beans. The reason is that Cr(VI) has a high oxidative power, damaging the cell membrane.

According to the US Environmental Protection Agency (USEPA) regulations, the concentration of hexavalent chromium in water should be less than 0.1 mg/L (Mortazavian *et al.*, 2016).

In the presence of certain minerals, such as manganese oxides, the oxidation of Cr(III) to Cr(VI) in the soil is promoted (Salem *et al.*, 1989), increasing the risk of water contamination due to its mobility (Haroun, Idris and Omar, 2009). In contact with organic matter, Cr(VI) is reduced to Cr(III), but if it is present at high concentrations, the reducing capacity of the environmental conditions can be exceeded (Devars *et al.*, 2001).

Chromium (III) is a hard acid and tends to form complexes with a variety of ligands such as water, ammonia, urea, halides, sulfates, ethyl diamine, and organic ligands containing oxygen, nitrogen, or sulfur donor atoms. Approximately 50 percent of water-soluble Cr(III) is organically bound in neutral and basic solutions, it forms polynuclear compounds in which the chromium atoms are bound with OH or O bridges (Salem *et al.*, 1989).

Considering the tanning industry, Cr(III) can form several strong bonds with oxides, hydroxides, organic matter, and carbonates, commonly found in these wastes (Kokkinos, Proskynitopoulou, and Zouboulis, 2019).

Several studies hypothesize that the speciation of each metal in tannery sludge depends on its initial chemical state, the adsorption and precipitation mechanisms of the sludge, the stabilization of the material, and the humification process (Haroun, Idris, and Omar, 2009).

The attempt to recover energy and valuable by-products from tannery sludge is a procedure recommended by EU guidelines and aligns with the principles of the circular economy (BAT), with a focus on the high chromium content (https://eur-lex.europa.eu/eli/dec_impl/2013/84/oj).

1.3 Zeolites

Zeolites are crystalline aluminosilicates and hydrates containing alkali and alkaline earth metals (Na, K, Mg, Ca, Ba, Sr). The structure consists of an infinite repetition of SiO and AlO tetrahedra bonded together by shared oxygens atoms (Kesraoui-Ouki, Cheeseman, and Perry, 1994).

The substitution of silicon atoms with aluminum atoms leads to electrochemical instability that is balanced with cations. This instability gives zeolites their ion exchange capacity. Furthermore, the three-dimensional structure of zeolites has large internal cavities and channels (Novembre *et al.*, 2022).

Natural zeolites are porous and characterized by their ability to reversibly absorb or release water, they can adsorb molecules and exchange ions without changing their initial structure (Montalvo *et al.*, 2012). The exchangeable ions are relatively harmless, and this characteristic makes zeolites interesting for the removal of a series of compounds from industrial effluents, including heavy metal ions (Kesraoui-Ouki, Cheeseman, and Perry, 1994).

Their use dates back as far as 2,000 years ago, when the Maya used zeolites as filters for water purification (Tankersley *et al.*, 2020), but it was not until the 1950s that scientists further investigated their physical and chemical properties (Kesraoui-Ouki, Cheeseman, and Perry, 1994). They are present in different parts of the world, especially near active or extinct volcanoes (Montalvo *et al.*, 2012).

The increasing accumulation of heavy metals in water, also due to the large and growing number of industrial activities that dispose of them in wastewater, has led to research into materials and methods for their treatment and removal. Natural zeolites are promising materials for removing heavy metals (Velarde *et al.*, 2023).

Specifically, the interest in natural zeolites is not only due to their non-toxicity but also to their easy availability, low cost, and excellent physical and chemical properties. These include thermal and chemical stability, and ion exchange capacity (Rodríguez-Iznaga, Shelyapina and Petranovskii, 2022).

This selectivity in ion exchange is confirmed for clinoptilolite, phillipsite, chabazite, and other zeolites, which show a high affinity for the NH_4^+ ion (Novembre *et al.*, 2022). There are more than 30 natural zeolites but only seven: chabazite, clinoptilolite, eronite, ferrierite, philipsite, mordenite, and analcime, are present in abundance to be considered exploitable natural resources (Kesraoui-Ouki, Cheeseman and Perry, 1994). Different types of zeolites differ due to the ways the tetrahedra bond in space and the type of ions they replace in the interstices (Kesraoui-Ouki, Cheeseman, and Perry, 1994).

Clinoptilolite is a common and widely used zeolite. Its low cost, availability, and ion exchange properties make it a good candidate for using and creating new functional materials (Rodríguez-Iznaga, Shelyapina, and Petranovskii, 2022). Due to its negatively charged lattice, it is already used in water treatment for the removal of heavy metal ions through ion exchange (Rodríguez-Iznaga, Shelyapina and Petranovskii, 2022; Kurniawan *et al.*, 2023).

1.4 Anaerobic digestion

Anaerobic digestion is an interesting and popular process for managing organic waste from different sources (L. De Baere, 2013a). It allows waste to be stabilized, biogas and organic acids to be recovered and total solids to be reduced. It is a process in which anaerobic bacteria work in synergy, resulting in different metabolic pathways, transforming complex organic matter into value-added products (Li, Park, and Zhu, 2011). Anaerobic digestion consists of four successive steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Meegoda *et al.*, 2018) shown in Figure 2.

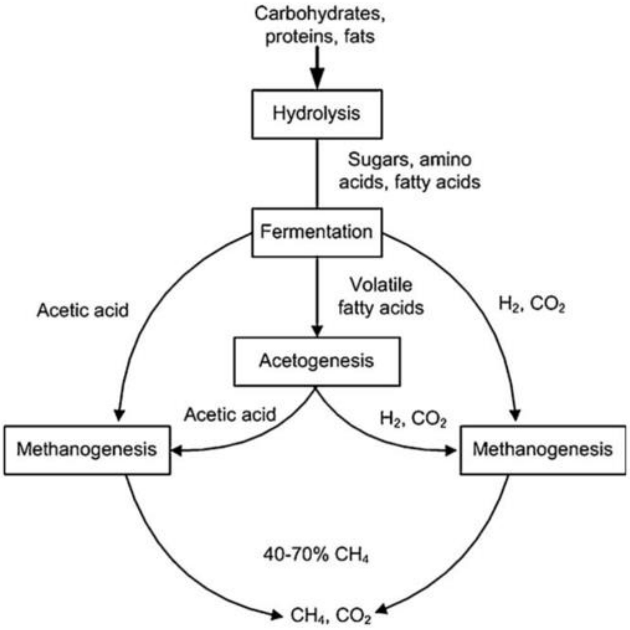


Figure 2. Simplified scheme of the anaerobic digestion process (Li, Park and Zhu, 2011).

Hydrolysis transforms complex polymers into simpler, soluble molecules that are available to acidogenic bacteria. Proteins, lipids, and carbohydrates are converted into amino acids, long-chain fatty acids, and sugars through the action of extracellular enzymes released by hydrolytic bacteria (Li, Park, and Zhu, 2011).

Then acidogenic bacteria absorb the hydrolysis products and convert them into short-chain fatty acids (Meegoda *et al.*, 2018) and other products such as carbon dioxide, and hydrogen (Li, Park, and Zhu, 2011). The concentrations of these products may depend on the conditions in the digester (Meegoda *et al.*, 2018). The next step is acetogenesis in which acetogenic bacteria convert organic acids and other intermediates into new substrates for methane production. These substrates are acetate, carbon dioxide, and hydrogen (GERARDI, 2003).

Finally, methanogenesis occurs in which methanogenic bacteria produce methane using the substrates previously produced during acetogenesis (Ferry, 2010). This study focuses only on the earlier stages of methanogenesis, in particular the process of anaerobic fermentation with the production of volatile fatty acids.

The application of this procedure to tannery sludge is difficult due to its complex composition (Mannucci *et al.*, 2010). Some difficulties are encountered due to the high protein component that influences the biomass composition, the slow hydrolysis rate, and the formation of sludge granules (Mannucci *et al.*, 2010).

1.5 Short chain fatty acids (SCFAs)

The International Union of Pure and Applied Chemistry (IUPAC) defines fatty acids as "carboxylic acids with an aliphatic tail." Fatty acids are categorized based on the length of their aliphatic carbon tails, which give them unique chemical properties. Short-chain fatty acids (SCFAs) (Table 1) are carboxylic acids that contain aliphatic tails of fewer than six carbon atoms (Layden *et al.*, 2013). They are highly soluble in water and decrease in quantity as the number of carbon atoms in the aliphatic chain increases (Volker et al., 2014). SCFAs include formic acid (C1), acetic acid (C2), propionic acid (C3), isobutyric acid (C4), butyric acid (C4), isovaleric and valeric acid (C5), and 2-methylbutanoic acid (C5) (Layden *et al.*, 2013). Today, the production of these compounds employs chemical synthesis using non-renewable materials such as crude oil (Volker *et al.*, 2014).

They can also be produced by anaerobic digestion and are of great commercial interest that has intensified with the rising cost of oil and the prospect of reducing the use of non-renewable petrochemicals (Akaraonye, Keshavarz, and Roy, 2010).

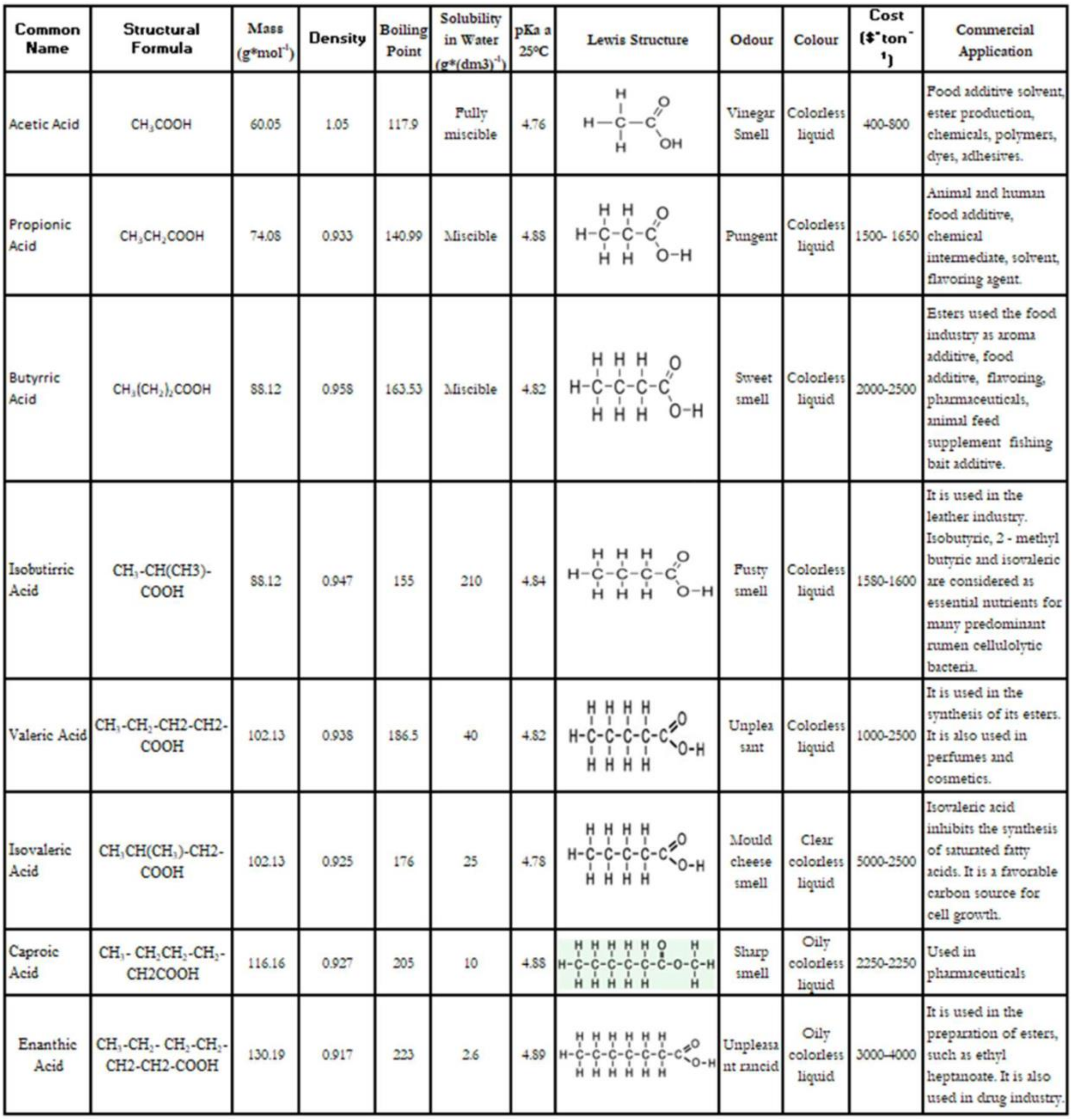
SCFAs can be used as substitutes for certain elements in the chemical industry or as raw materials for the production of chemicals (Valentino *et al.*, 2021). They are potential sources of renewable carbon and could be used to produce biofuels, pharmaceuticals, cosmetics, food additives, and polymers. In particular, SCFAs and lactic acid are direct precursors of polyhydroxyalkanoates (PHAs) (Kourmentza *et al.*, 2017). These biopolymers can replace some synthetic materials due to their biodegradability and thermoplastic properties (Valentino *et al.*, 2017). The most common SCFAs produced in acidogenic fermentation are acetic, propionic, and butyric acid (Zang *et al.*, 2022).

The production of SCFAs from organic material is an option that raises ethical concerns due to the use of food substances to produce chemicals (Kondo and Kondo, 1996; Zigova, 1999). To overcome this problem, processes have been developed to obtain them from the organic fraction of waste (Bengtsson *et al.*, 2008).

The production of SCFAs from the acidogenic fermentation of organic waste is influenced by several factors such as pH, temperature, total solids (TS), and retention time. The analysis of these factors showed that their values strongly depend on the characteristics of the specific substrate (Lee *et al.*, 2014).

These parameters will be further explained in the upcoming chapters.

Table 1. Chemical and physical properties of SCAFs, their economic value, and commercial applications (Zappalorto, 2021).



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_3COOH	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_3CH_2COOH	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	$CH_3(CH_2)_2COOH$	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	$CH_3CH(CH_3)$-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_2CH_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.

1.5.1 pH

According to Lee et al., 2014, pH values between 5.25 and 11 allow the production of SCFAs, however, the optimal ones vary depending on the type of waste. For wastewater, acidic pH values are preferred, while for municipal waste, alkaline pH values are preferred (Lee *et al.*, 2014). Most acidogenic bacteria do not survive in environments lower than pH 3 or higher than pH 12, which is why pH values must be kept in the ideal range for their survival (Liu *et al.*, 2012). According to the study by Gottardo et al. 2017 on food waste, the fermenter must maintain pH values above 5 so that fermentation is possible but not above 7 because it would cause methanogenic bacteria to proliferate. Hydrolysis of sludge improves in alkaline conditions, ionizing the carboxyl groups of carbohydrates and proteins, resulting in their release into the environment (Liu *et al.*, 2012).

In contrast, for kitchen waste, the optimal conditions for hydrolysis and acidogenesis are at pH 7 (Zhang *et al.*, 2005). Furthermore, the type of SCFAs produced can be influenced by pH, in particular acetic, propionic, and butyric.

According to a recent study by Tuci et al. 2022 on tanneries, it was noted that despite an increase in SCFAs values during the acidification process, pH values remained stable around 7. The authors attributed this lack of pH variation to the presence of lime ($Ca(OH)_2$), which acts as a buffer by releasing OH^- ions. This means there was no need to adjust the pH value during the experimental phase. Lime is commonly used in the "Liming" process for hair removal and fiber bundle removal, as well as a pH corrector for wastewater undergoing the primary sedimentation process.

1.5.2 Temperature

Temperature is a factor that influences the growth of microorganisms, enzymatic activity, and the rate of hydrolysis (Zhou *et al.*, 2018).

Short-chain fatty acids (SCFAs) can be produced at different temperature ranges, namely psychrophilic conditions (4-20°C), mesophilic conditions (20-50°C), thermophilic conditions (50-60°C), and extreme/hyperthermophilic conditions (60-80°C) (Lee *et al.*, 2014).

The study by Hao and Wang 2015 states that SCFAs production increases tenfold when fermentation occurs under thermophilic conditions, as opposed to mesophilic conditions, and without pH regulation. At 60°C, biological acclimation may be better, and acidogenesis is more active compared to mesophilic temperatures (Mengmeng *et al.*, 2009).

Furthermore, according to Zhang et al., 2009, thermophilic conditions increase the hydrolysis rate, leading to an increased SCFAs production. However, under thermophilic conditions, microorganisms are more sensitive to environmental changes compared to mesophilic conditions (Kim *et al.*, 2006).

In the literature, the results about the ideal temperature are scarce, possibly due to the presence of different microbial species and different substrates (Lee *et al.*, 2014). An example is the study by Yu et al., 2013 which claims that there was no effect on SCFAs production when treating activated sludge within the temperature range of 45-70°C. However, according to Zhuo *et al.*, 2012 under thermophilic conditions (55°C), the activity of enzymes responsible for acid formation is lower than under mesophilic conditions (37°C). SCFAs production decreased by 40% when shifting from 37°C to 55°C.

However, the choice of temperature condition has to be made from an energy-saving perspective to find a compromise between the SCFAs production and the heat required to maintain the temperature (Yu, Fang, and Gu, 2002). Temperature also influences the type of SCFAs produced, at 55°C, primarily butyrate is produced, while at 35°C, propionate and acetate are produced from food waste (Jiang *et al.*, 2013).

Similar to pH, the optimal temperature also varies depending on the substrate (Valentino *et al.*, 2019). Transitioning from psychrophilic to mesophilic conditions increases the SCFAs concentration (Zhang et al., 2009; Yuan, Sparling, and

Oleszkiewicz, 2011), the SCFAs production rate (Maharaj and Elefsiniotis, 2001), and yield (Bouzas *et al.*, 2002). In the study by Zhang et al., 2009, it was observed that the concentration of SCFAs produced from waste-activated sludge increased by 300% raising the temperature from 10°C to 35°C. This indicates that an increase in temperature leads to improved hydrolysis and, therefore, an increase in the number of soluble carbohydrates (Zhang *et al.*, 2009).

Soomro et al., 2020 treated a mixture composed of an organic fraction of municipal solid waste and activated sludge at 37°C and 55°C to produce biogas and SCFAs. Under mesophilic conditions, the production was 20g COD_{SCFA}/L, whereas, under thermophilic conditions, it ranged from 16.5 to 31.6g COD_{SCFA}/L.

1.5.3 Total Solids (TS) and Total Volatile Solids (TVS)

The concentration of total solids (TS) is another parameter that influences the efficiency of an anaerobic process. Two types of processes are identified according to TS content: wet digestion for TS <15% and dry digestion for high-solids TS >15-20% (Karthikeyan and Visvanathan, 2013; L. De Baere, 2013b). A high TS content allows the use of a smaller reactor, leading to lower energy consumption (Motte *et al.*, 2013). Higher TS content releases more nutrients, allowing bacteria to produce more SCFAs (Liu *et al.*, 2018). In contrast, according to Battista et al., 2018, a high TS content can alter sedimentation properties and influence other rheological properties of the medium such as viscosity, overall decreasing microbial community activities, and metabolic pathways. It can be deduced that there is a threshold of TS content for efficient SCFAs production. This threshold would depend on the rheology and mass transfer of the system (Liu *et al.*, 2018).

1.5.4 Hydraulic retention time (HRT)

According to the literature, the best HRT value depends on the type of substrate. However, it can also vary with the same substrate (Atasoy *et al.*, 2019).

A high HRT value can positively influence the production of short-chain fatty acids (SCFAs) in anaerobic fermentation processes. This is because a longer HRT allows microorganisms more time to react with the waste, generating SCFAs (Lee *et al.*, 2014).

As stated by Lim et al., 2008 the production of SCFAs from fractionated organic waste significantly increased when HRT was extended from 2 to 6 days. However, it is important to note that an excessively prolonged HRT may result in stagnant SCFAs production (Lee *et al.*, 2014). In addition, to implement treatments with high HRT values, reactors with larger volumes would be required, leading to increased costs (Demirel and Yenigun, 2004).

For example, in the context of dairy wastewater treatment, an increase in HRT from 4 hours to 12 hours almost doubled the production of SCFAs, but further increases to 16-24 hours resulted in only a modest improvement of 6% (H.P.Fang, 2000).

This same principle also applies to other cases, such as the acidogenic fermentation of food waste (Lim *et al.*, 2008) and the co-fermentation of sewage sludge and fruit/vegetable waste (Dinsdale *et al.*, 2000). In both cases, an increase in HRT led to an increase in the concentration of SCFAs up to a certain point, but further prolongations of HRT did not lead to significant improvements in the production of SCFAs (Lee *et al.*, 2014).

An interesting example is provided by the research conducted by Bolaji and Dionisi, 2017 who studied the anaerobic fermentation of vegetable waste with HRT of 10, 20, and 30 days. Their results showed that an increase in retention time improved the reduction of volatile solids and influenced the composition of SCFAs, with caproate appearing after 20 and 30 days, in addition to the main products of butyrate and acetate. In summary, HRT is a crucial parameter in the production of SCFAs, influencing both the quantity and composition of the products. However, it is crucial to strike a balance between HRT and the associated costs, precisely adjusting the retention time to maximize the economic and environmental benefits of anaerobic fermentation processes (Lee *et al.*, 2014).

1.6 Pre-treatment

As mentioned above, tannery sludge has a high concentration of proteins, which results in lower availability of the organic components. This can represent an obstacle to the efficiency of the anaerobic digestion process (Zhai *et al.*, 2020). Therefore, various pre-treatment methods have been explored to increase the solubilization of solid waste.

Improving the hydrolysis of solid waste is a goal often achieved through chemical pretreatment, which utilizes several common reagents such as acids, alkalis, ozone, and hydrogen peroxide (Lee et al., 2014).

Another option to improve the hydrolysis of solid waste is micro pretreatment (Appels *et al.*, 2013), which exploits thermal and athermal effects.

Another possibility is thermal pretreatment, which can be performed between 60 and 180°C (Climent *et al.*, 2007). The study by Appels et al., 2010 shows that thermal treatment of sludge solubilizes organic compounds efficiently. The greatest substance release occurs with a sixty-minute treatment at high temperatures (90°C), while the efficiency decreases slightly at 70°C.

2 Thesis purpose

The objective of this studio is to employ anaerobic digestion to utilize these waste materials for the production of high-value substances such as short-chain fatty acids (SCFAs), achieving economically and technically feasible concentrations and yields for their subsequent extraction. Another important goal of this study is the utilization of natural zeolites, employed to explore their capacity for removing dissolved chromium in the liquid phase, as well as removing ammonia, soluble organic matter (sCOD), and monitoring the efficiency of SCFAs production with the use of these new substances. The implementation of these solutions would contribute to reducing the amount of sludge in landfills, currently classified as hazardous special waste, with their only destination being controlled landfills.

The influence of pH, concentration of dissolved organic matter, and the presence of volatile solids (VS) on the production of SCFAs and their composition was investigated. Additionally, the capabilities of the zeolites used for the removal of chromium, initially bound to organic matter, and ammonia were analyzed. Specifically, the chosen values for these parameters were:

- pH: no substances were added to vary the pH values in this work, which remained within the range of 6.5-7.9.
- Temperature: acidogens are responsible for the production of SCFAs, particularly thermophilic bacteria (45–65°C) (Patel et al., 2021). This study involved the production of SCFAs from tannery sludge in thermophilic environments (55 ± 1°C).
- Total solids: the concentration of total solids (TS) in the sludge treated and used in this context is 70 g TS/L. Elevated TS levels are assumed to correlate

with higher concentrations of volatile solids (VS), indicating a potential increase in short-chain fatty acids generation.

- Thermal pretreatment: a thermal pretreatment was conducted as described later, to solubilize most of the organic matter present in the sludge. The parameters used were chosen based on the available instrumentation and values indicated in the studies of Climent et al. 2007 and Appels et al. 2010.
- Zeolites: the zeolites employed in this study were Chabazite and Clinoptilolite. The doses added to the reactors were 5g for reactors with a Hydraulic Retention Time (HRT) of 4 days, and 3g for reactors with an HRT of 8 days. The specific reasons for these quantities are detailed later.

3 Materials and methods

3.1 Feedstock (tannery sludge)

The tannery sludge (Figure 3) treated for these experiments comes from the WWTP in Montebello Vicentino in the province of Vicenza, Italy. This plant collects wastewater from several tanneries in the area. The plant treats about 10000 m^3/d of wastewater produced by 23 tannery plants. The wastewater from the tanneries undergoes primary treatment and sedimentation and secondary biological treatment. The quantity of total solids (TS) in the dried sludge, subsequently treated and used in this context, represents 84.4% by mass of TS and 59.4% by mass of volatile solids (VS).

Figure 3. Dry sludge collected from the Montebello Vicentino WWTP.

3.2 Feedstock pre-treatment

The dried sludge was subjected to mild thermal pre-treatment using Nautilus-Anaero Technology, shown in Figure 4, and then used to feed the reactors.

It consists of 15 1-litre reactors (plastic bottles) into which the substrate to be treated is loaded and placed in the water bath that maintains a stable temperature for the reactors. A lid with a reducer is then placed to align the reactors. A single mixing motor is used to ensure uniform mixing intensity for all reactors. In addition, the reactors are equipped with a gas flow measuring tube that can be connected to monitor biogas production.

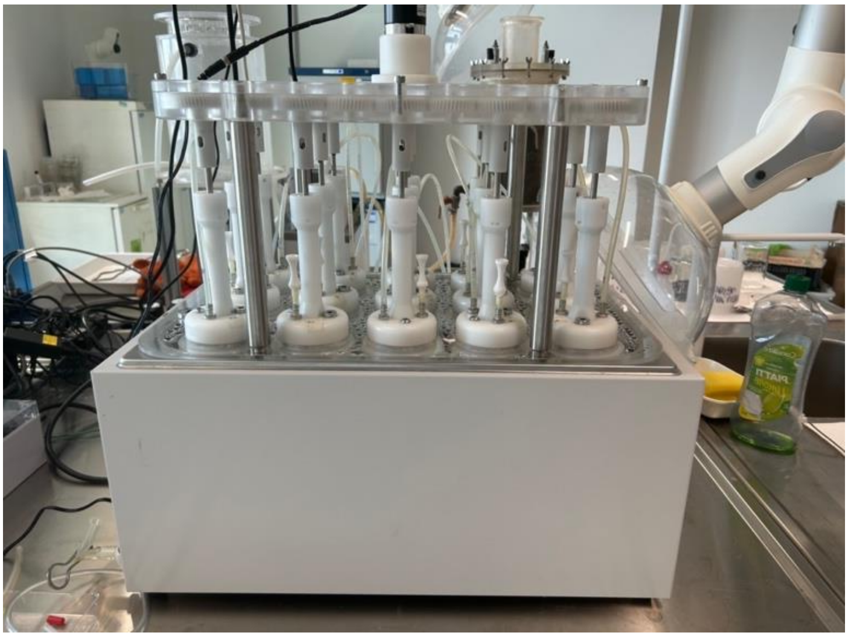


Figure 4. Nautilus device for the pre-treatment procedure of the feedstock.

In the plastic bottles of Nautilus-Anaero Technology, 85g of dried sludge was diluted in approximately one liter of tap water (S/I ratio of 7.0 w/w VS basis). The mixture was continuously stirred and kept at a constant temperature using a thermal bath at 70 degrees for 20 hours. The resulting sludge was stored in plastic canisters in a refrigerator.

3.3 Acidogenic fermentation test

The study involved the treatment of tannery sludge in two semi-continuously stirred reactors maintained under thermophilic conditions (Figure 5), with two different HRTs (4 days and 8 days). The selection of the HRT values for 4 and 8 days was based on the observation of the SCFAs concentration trend in a previous experiment conducted on the substrate by Tuci et al., 2022. In addition, the choice of these values was made with consideration of the fact that larger reactor volumes are linked to high HRTs. As a result, a lower HRT value was chosen to ensure a cost-effective strategy for potential full-scale use. Initially, 130 g of dried sludge and 1.5 l of tap water were added to the two reactors, the TS concentration obtained after dilution of the dry sludge was 7%. The mixture was kept in constant agitation and at 55 degrees for one week, after which the tests started.

Figure 5. Semi-continuous reactors for the tannery sludge fermentation.

Each cycle involved the fermentation of the sludge in the two reactors for 45 days. Every day, 375 ml and 189 ml (according to the chosen HRT) of sludge were taken from reactors A (HRT 4) and B (HRT 8), respectively, and the same quantities of pre-treated sludge stored in the refrigerator were fed in each reactor.

Three cycles were carried out. The first cycle involved the use of pre-treated sludge only. The second and third cycles involved the addition of two different zeolites (Figure 6). The dosage chosen for the zeolite was 0.26g/gVS, as it provided the most fermentation products (Silva *et al.*, 2021) and the best uptake of heavy metals (Zorpas *et al.*, 2002).

During the second cycle, 3g in reactor A and 5g in reactor B of chabazite were added. The same was done in the third cycle using clinoptilolite previously pulverized with a ceramic mortar.

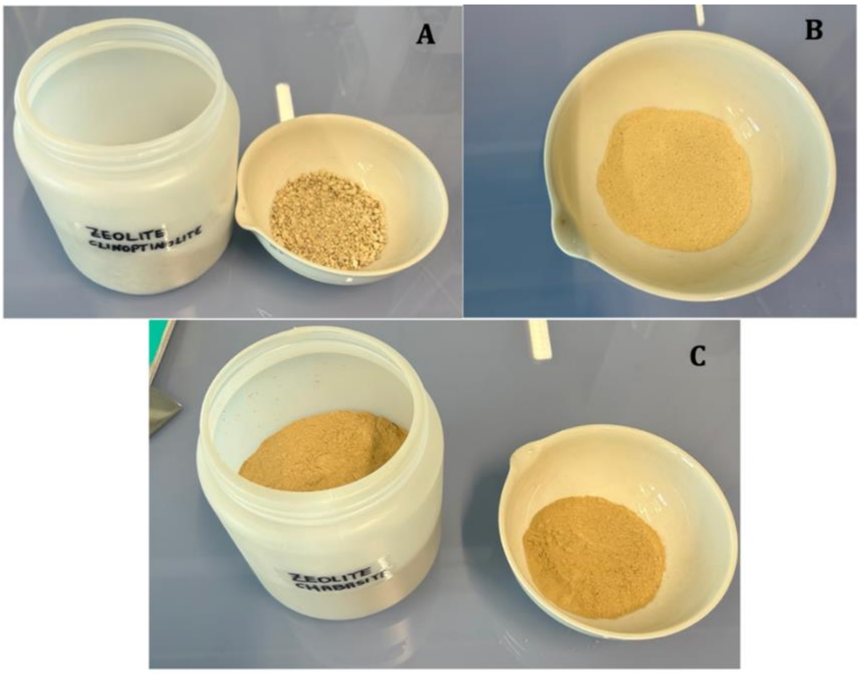


Figure 6. Zeolites. A: clinoptilolite, B: pulverized clinoptilolite, C: chabazite.

3.4 Sampling

3.4.1 Analysis of SCFAs, SCOD, and ammonia

Two or three times a week, samples of the sludge were taken to be used for SCFAs, ammonia, and sCOD analysis. The withdrawn samples were then centrifuged at 9000 rpm for 30 minutes and filtered at 0.2μm.

3.4.2 Chromium analysis

Chromium sampling was carried out twice a week for the first 14 days and then once a week, and 120 ml of sludge was taken from each reactor. The analyses were subsequently carried out by an external company.

3.5 Analyzed parameters

3.5.1 pH

Before each sampling, the pH was measured by immersing the pH meter probe (Figure 7) in the two reactors. The instrument was calibrated monthly with three solutions of known pH (4,7,10).

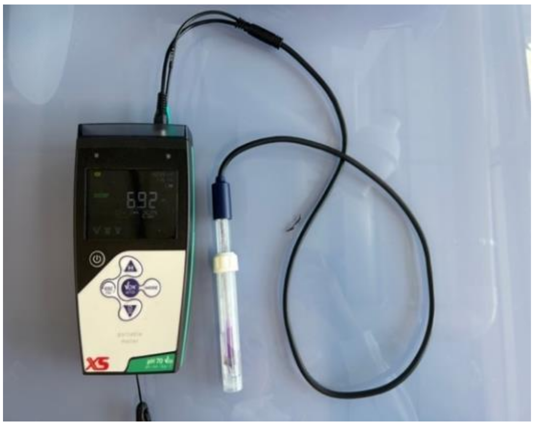


Figure 7. pH meter used for analysis.

3.5.2 TS and TVS

For each reactor, 40 ml of sludge was taken from the reactors and placed in two previously weighed empty crucibles. The crucibles containing the sludge were then weighed and placed in an oven for 48 hours at 105°C and finally weighed. This procedure removes all the water in the sample and the residue obtained after complete evaporation represents the Total Solids (TS) content in the sample.

The formula used to quantify TS as gTS/Kg was:

$$TS = \frac{T_2 - T_0}{T_1 - T_0} \times 1000$$

T0 weight of empty crucible.

T1 wet weight of crucible with sample.

T2 dry weight of crucible with sample.

Subsequently, the crucibles were placed in an oven at 550°C for 24 hours and then weighed. This allowed the determination of the volatile suspended solids (TVS) representing all those undissolved substances present in the sample.

$$TVS = \frac{T_2 - T_3}{T_1 - T_0} \times 1000$$

T0 weight of empty crucible.

T1 wet weight of crucible with sample.

T2 dry weight of crucible with sample.

T3 dry weight of crucible with sample after muffle furnace.

3.5.3 sCOD

The analysis is based on a colorimetric method, using a spectrophotometer (Figure 8) following Standard Methods (Apha Awwa, 2005).

Figure 8. A spectrophotometer was used for the analysis.

Soluble chemical oxygen demand (sCOD) is a fraction of COD that quantifies soluble organic substances (Zhang et al. 2009). To perform the analysis the samples were diluted and then placed in the spectrophotometer tubes. The blank consisted of distilled water. Next, the oxidizing solution, $K_2Cr_2O_7$, and the catalyzing solution,

H_2SO_4, were added. The tubes then remained in the oven for 120 min at 150°C. Once cooled, the absorbance of the samples was then measured with the spectrophotometer set at 600 nm. Before measuring the samples, the instrument had to be calibrated with the blank. The amount of sCOD was determined using the following formula:

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

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

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

3.5.4 Ammonia

Ammonia nitrogen analyses were performed with an ISE selective electrode (Figure 9). This electrode (Ion selective electrode) is selective towards ammonia in gaseous form thanks to a polytetrafluoroethylene (PTFE) membrane.

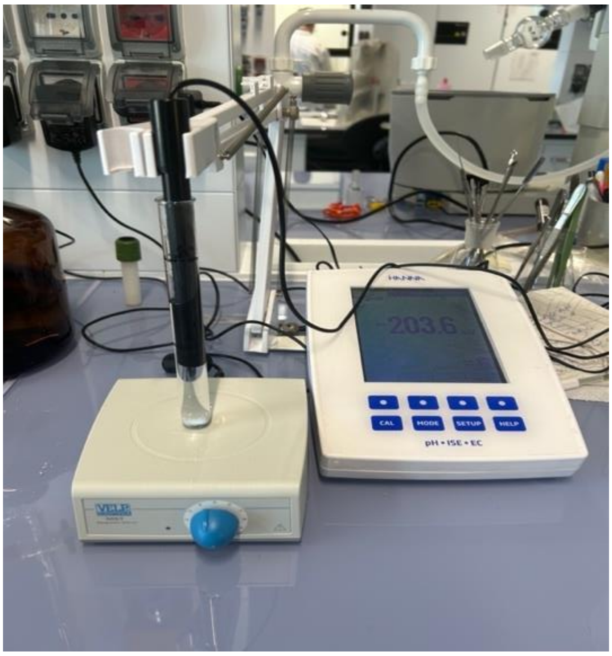


Figure 9. ISE selective electrode used for analysis.

The previously thawed samples were diluted 1:10 with distilled water. Solutions for calibration were prepared by performing a serial dilution with a 0.1M ammonia standard solution and distilled water (0.1-0.01-0.001-0.0001-0.00001M).

The probe was set in mV and starting with the least concentrated solution the calibration was performed. The measurement involved immersing the probe in the sample contained in the tube, which was kept in continuous agitation through a stirring rod and to which 0.2μL of ISA solution was added. Once the value in mV was stable it was recorded on an Excel file. The calibration involved a graph showing the recorded mV values and the respective concentrations reported as -log. The calibration line and R^2 coefficient were derived.

The ammonia values in the samples were calculated using the formula:

$$NH_4^+(\frac{mg}{L}) = 10^{-\frac{mV-q}{m}} \times MM_{NH_4^+} \times FD \times 1000$$

Where: “mV” is the recorded potential value, “q” is the intercept of the calibration line, “m” is the angular coefficient of the calibration line, $MM_{NH_4^+}$ is the molecular mass of ammonium.

3.5.5 SCFAs

The concentration of SCFAs was determined using a Gas Chromatograph (GC), Agilent 6890N series with an Agilent J&W DB-FFAP column (length 15 m, diameter 0.530 mm, film 1.00 μm) coupled with a FID flame ionization detector (Figure 10), the calibration was done in 3 points using a stock solution containing the target short-chain fatty acids and 2-ethylbutyric acid was used as an internal standard for the quantitative analysis.

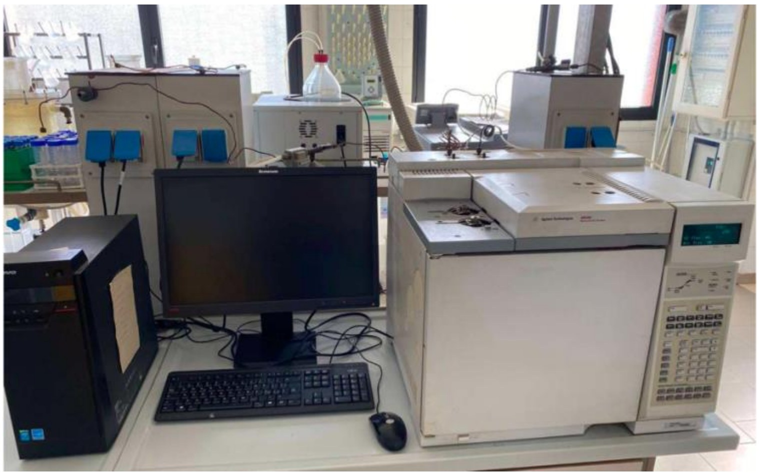


Figure 10. Gas chromatograph used for SCFAs quantification.

The method involves a temperature ramp increasing from 80° C, to 200°C and ending with a post-run phase at 220°C. The sample was previously subjected to filtration at 0.2 μm to avoid clogging the capillary column. The concentrations of these SCFAs are determined by comparing the peak area in the chromatogram with that of the internal standard; for this reason, 2-ethylbutyric acid must always be present in a known quantity in both the samples and the standards used in calibrating the instrument. The target acids for analysis are acetic acid (C2), propionic acid (C3), isobutyric acid (iC4), butyric acid (C4), isopentanoic acid (iC5), pentanoic acid (C5), isocaproic acid (iC6), caproic acid (C6) and heptanoic acid (C7).

3.5.6 Chromium

Hexavalent chromium Cr(VI) was analyzed in the liquid phase at the end of each test to quantify any possible release of Cr(VI). An external laboratory performed the analyses. Hexavalent chromium was determined calorimetrically by reaction with diphenylcarbazide in an acid solution (pH 1.6-2.2), achieved by adding a certain amount of H_2SO_4. Adsorption was measured at a wavelength of 540 nm.

4 Results and discussion

In the following discussion of the results, the series are indicated by the following abbreviations:

- T4: Zeolite-free reactor with HRT4
- T8: Zeolite-free reactor with HRT8
- CHA 4: Chabazite-treated reactor with HRT4
- CHA 8: Chabazite-treated reactor with HRT8
- CLI 4: reactor treated with HRT4 clinoptilolite
- CLI 8: reactor treated with clinoptilolite with HRT8

4.1 Effect of the pH on the Tannery Sludge Fermentation

Approximately after 7-12 days, the period of stability of the reactors is reached and the pH values for this period show an average range of 7.2-8 without significant variations (Figure 11). Higher pH values are evident in the reactors in which zeolites were added (7.5-8) than in the series without zeolites (7.3-7.2). It can be seen that in the T4 and T8 series, pH values gradually increase over time, with the T4 series starting from pH 6.5 up to pH 7.7 and the T8 series starting from pH 6.7 up to pH 7.5. Unlike the other series with zeolites, the CHA 4 series shows a slight upward trend in pH values from a low of 7.16 up to 7.93, this may be due to the shorter residence time that did not allow the zeolite to exert its buffer function as in the other series. In contrast, the remaining zeolite-treated series does not show a gradual increase in pH, which lies around an average range of values of 7.5 for CHA 8, 7.6 for CLI 4, and 7.6 for CLI 8.

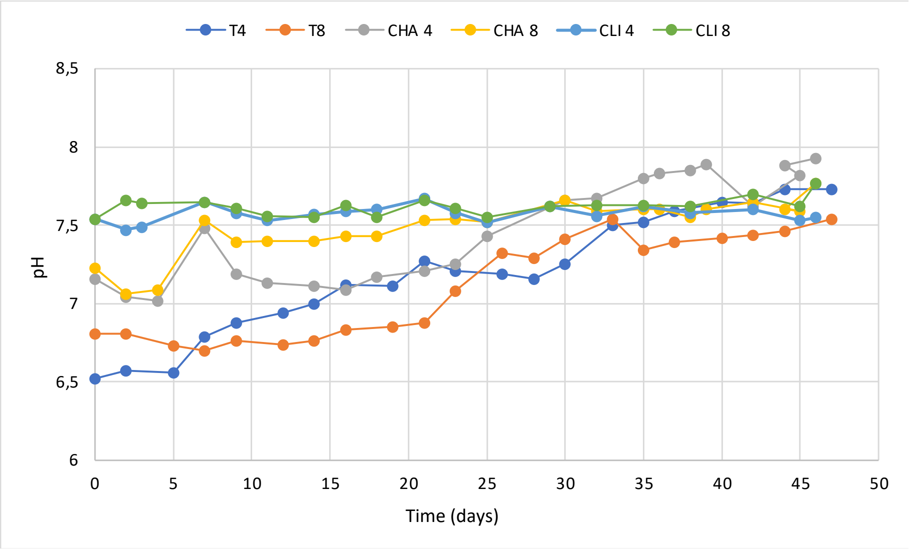


Figure 11. pH trends were observed in all tests performed.

The maintenance of these values is attributable to the lime-containing sludge, which releases OH^- ions acting as a buffer and pushing the pH towards neutrality. Lime is present because it is used in the 'liming' process in which fiber bundles are removed from the raw hides. This peculiarity allows the process to proceed without the need for an external pH correction. Furthermore, it can be assumed that zeolite contributed to the stable maintenance of pH values. This also emerged in the Castellar et al. 1998 study in which the zeolite acted as a pH buffering agent, keeping it stable during fermentation. Finally, looking at Figure 12 below, it is evident that there is no significant influence of pH on acidification performance.

4.2 Production of SCFAs over time

Figures 12 and 13 show a similar trend in SCFAs production in all series, with rapid growth until a plateau is reached.

The T4 series reaches the plateau after 9 days, while the T8 series reaches the plateau on day 12. Both sets with chabazite plateau on day 9, while the CLI 4 series plateau before all other sets on day 7, and the CLI 8 series plateau on day 11.

Examining the period required to reach the maximum concentration of SCFAs is a key indicator of the full utilization potential of this sludge, focusing on the acidification of organic matter. This assessment involves choosing an appropriate hydraulic retention time (HRT) depending on the desired production, thus outlining the optimal strategy to maximize the effectiveness of the process.

Concerning the maximum concentration of SCFAs produced, the T8 and CHA 8 series recorded similar concentrations (18.7-22.1 $gCOD_{SCFA}/L$ and 18.8-21.7 $gCOD_{SCFA}/L$) and were the series with the highest results. In general, the HRT 8 tests show a higher production of SCFAs than the four-day retention time tests with mean values of 20.3, 20.2, and 17.1 $gCOD_{SCFA}/L$ for T8, CHA 8, and CLI 8, respectively. In contrast, the mean values of SCFAs produced with HRT4 were 16.7, 17.0, and 14.6 $gCOD_{SCFA}/L$ for T4, CHA 4, and CLI 4.

Tests performed with clinoptilolite show lower SCFAs production than those performed with chabazite or without the use of zeolites. This discrepancy could be associated with the structure of clinoptilolite, which may have absorbed some of the dissolved organic matter and SCFAs in its structure.

In general, the series containing zeolites show lower sCOD concentrations (shown in Table 2) than the tests performed without the addition of zeolites. This phenomenon could be attributed to the previously mentioned process in which part of the dissolved organic matter is absorbed into the zeolite structure (Montalvo *et al.*, 2012).

Furthermore, the levels of SCFAs recorded for the CLI 4 and CLI 8 series are lower than those found in the other series. Consequently, it is observed that the corresponding Y_F values are also lower.

In general, these conditions could be evaluated in the future for an industrial-scale development of the SCFAs production process from these sludges, with the difficulty, however, of the temperature conditions to be maintained requiring continuous heat. From the values obtained, it is stated that the zeolites had no significant impact on the production of SCFAs. The tests with the highest retention time, especially T8, produced the best results. This was expected because a longer residence time allows microorganisms to interact more with the substrate, increasing the production of SCFAs.

In the literature, there is a lack of studies on the production of short-chain fatty acids (SCFAs) from tannery sludge. The study conducted by Tuci and colleagues in 2022, also focusing on tannery sludges, reveals similar trends in SCFAs production, with a plateau reached around the ninth day for thermophilic tests, mirroring the findings of this study. The concentrations of short-chain fatty acids produced in this research are slightly lower than the values reported by Tuci in 2022 for sludges pre-treated with microwave-hydrogen peroxide and maintained under thermophilic conditions (23.7-26.2 $gCOD_{SCFA}/L$). However, they are higher than the concentrations obtained for sludges kept under thermophilic conditions but not pre-treated (7.1-7.4 and 13.9-15.3 $gCOD_{SCFA}/L$) in the same study.

Since the results of this study are similar to those obtained by Tuci in 2022, it can be concluded that the thermal pre-treatment was effective and practical. Moreover, it proved to be more economically advantageous as hydrogen peroxide, a chemical requiring a separate purchase, was not needed. Moreover, these results can be compared with other works on similar substrates such as municipal sludge from treated wastewater.

Lorini et al. 2022 examined SCFAs production from thickened sludge with a solids content of 30 gTS/KgWAS for 8-9 days (HRT), under mesophilic and thermophilic conditions. Namely, under thermophilic conditions, SCFAs production was 7.7 ± 0.3 $gCOD_{SCFA}/L$ and 8.8 ± 0.1 g COD_{SCFA}/L also at thermophilic temperature, but with a heat pretreatment of 70°C for 48 hours. These results show significantly lower values than those recorded in the present research. This suggests that lower contents of total solids, and possibly also of volatile solids (VS), correspond to lower availability of substrate for the conversion of organic matter into short-chain fatty acids (SCFAs). Thus, tannery sludge may be a promising substrate for acid production under the conditions adopted in this study.

The work conducted by Strazzera and colleagues in 2021 investigated the impact of different major carbon fractions in organic municipal solid waste (OFMSW) on the production profile of volatile fatty acids (SCFAs) by performing batch tests. The results showed a significantly high production of SCFAs in protein-rich OFMSW, reaching a final SCFAs concentration of almost 14 gCOD/L at pH 7. In contrast, lower yields of SCFAs were recorded in OFMSW fractions containing sugars, fibers, and lipids. Furthermore, in this study, the use of protein-rich MSW showed higher yields of short-chain volatile fatty acids (SCFAs), results that were just below those obtained for the T4, CHA 4, and CLI 4 series in this research. A common element between these could be the abundant presence of proteins in both substrates, which favors a higher production of SCFAs compared to other organic carbon fractions.

It should be noted that a sugar-rich fraction exhibits high hydrolysis kinetics, leading to rapid acidification of the reaction medium and inhibition of short-chain fatty acid-producing microorganisms. Conversely, in a lipid-rich environment, the presence of numerous long-chain fatty acids is observed, which damage cell membranes, reduce cell permeability and, consequently, hinder nutrient transport (Fernández-Domínguez *et al.*, 2020).

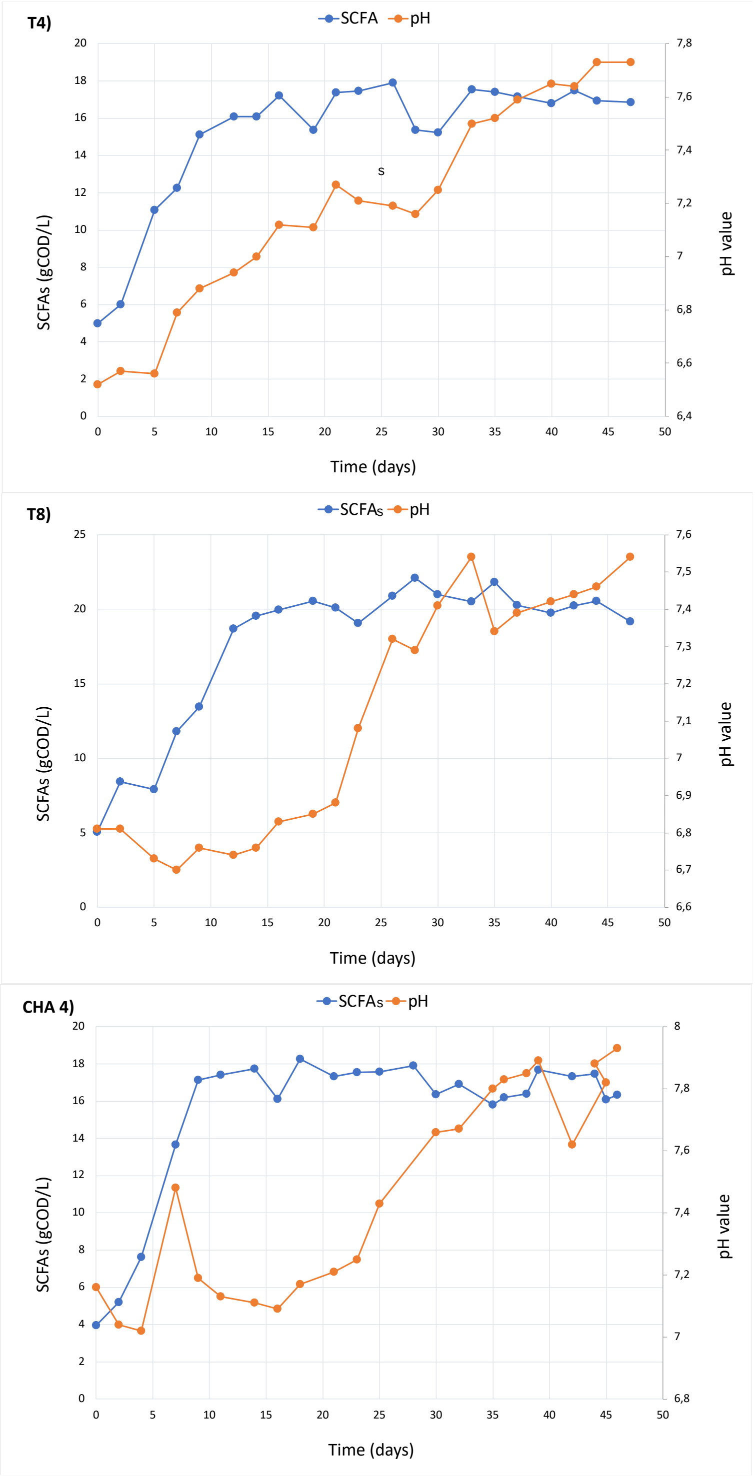


Figure 12. Concentrations and trends (gCOD/L) of SCFAs in the T4, T8, and CHA 4 tests.

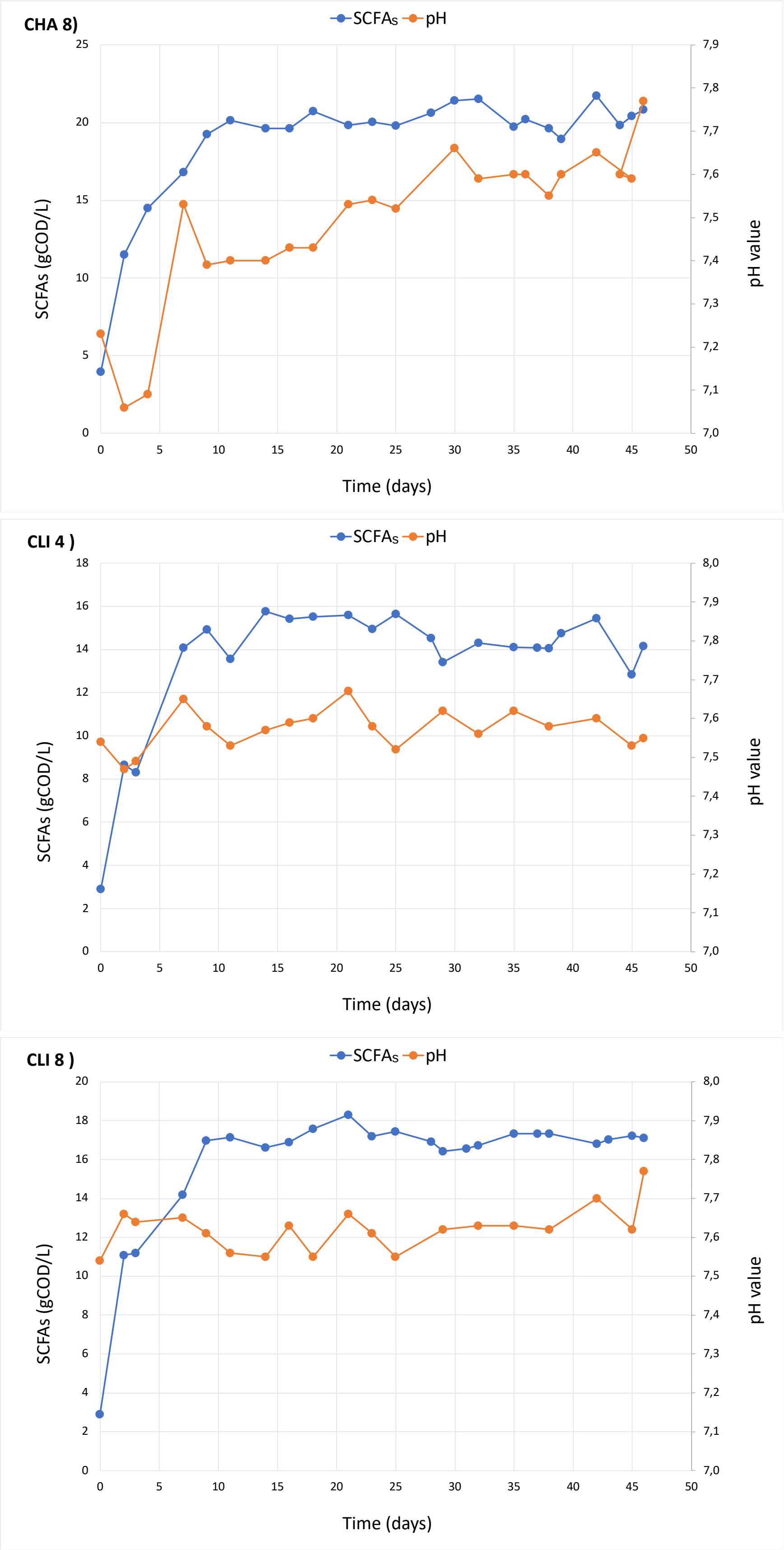


Figure 13. Concentrations and trends (gCOD/L) of SCFAs in the CHA 8, CLI 4, and CLI 8 tests.

4.3 SCFAs/COD_{SOL} Ratio

Table 2 shows the sCOD values in all series. The highest values are associated with the tests with the longest retention time (23±0.4 and 28±0.5 for T4 and T8, 19±0.2 and 22±0.3 for CHA 4 and CHA 8, and 20±0.4 and 23±0.2 for CLI 4 and CLI 8). The series treated with zeolites show lower values, this could be attributed to an absorption of soluble organic matter on the zeolite structure as mentioned above.

In addition, the gradual increase in soluble organic matter is associated with the degradation of organic products, which leads to a gradual increase in their solubilization and, consequently, the COD_{SOL} level. This increase is not always supported by a parallel acidification process, particularly when thermophilic conditions are used for certain types of substrates (Valentino *et al.*, 2021). Therefore, during the acidogenic fermentation process, it is crucial to maintain an adequate balance between the solubilization and acidification processes of organic matter. The SCFAs/COD_{SOL} parameter is a significant indication of the technical feasibility of the waste fermentation process, as it reflects the effectiveness of the conversion of soluble organic matter into SCFAs. Table 2 shows the results of these parameters obtained for each series considering the period of stability.

Table 2. SCFAs/CODSOL Ratio and sCOD and series standard deviation.

	T		CH		CLI	
	T4	T8	CHA 4	CHA 8	CLI 4	CLI 8
SCFAs/COD (gO_2/gO_2)	**0,72**	**0,72**	**0,87**	**0,92**	**0,71**	**0,75**
DS	0,044	0,050	0,045	0,030	0,056	0,022
sCOD (gO_2/L)	**23**	**28**	**19**	**22**	**20**	**23**
DS	0,4	0,5	0,2	0,3	0,4	0,2

The T and CLI series show values within the range of 0.71-0.75 gO_2/gO_2, the range of the CH series being higher with values of 0.87-0.92 gO_2/gO_2. These results indicate that more than 70% of the organic matter contained in tannery sludge has been converted to SCFAs, up to a maximum of 92% for the CHA 8 series, highlighting the increased conversion efficiency aided by the added Chabazite. These data are similar to those obtained in Tuci et al., 2022 on tannery sludge pre-treated with H_2O_2 and maintained under thermophilic conditions, with values obtained above

0.70 gO_2/gO_2, in contrast to the untreated sludge whose range remained lower (0.60-0.65 gO_2/gO_2).

Based on this, it is assumed that the pretreatment leads to an improvement in the thermophilic acidification process as COD_{SOL} becomes more available for conversion into SCFAs.

With other carbon sources such as the organic fraction of municipal solid waste (OFMSW), which contains a lot of putrescible organic matter compared to tanning sludge, SCFA/COD_{SOL} values reach up to 90% (Gottardo *et al.*, 2022), similar to those obtained in the CHA 8 series.

Finally, acidogenic fermentation of municipal sewage sludge shows lower results than in this work (0.51-0.69 gO_2/gO_2) (Presti *et al.*, 2021).

4.4 Fermentation Yield (Y_F)

Fermentation yield has an important relevance because it is linked to the amount of SCFAs potentially recoverable from the volatile suspended solids that represent a source of carbon. Quantifying this parameter is also necessary for a possible economic assessment of the biorefinery value chain. Table 3 shows the average Y_F of all series, calculated over the stable production period of the SCAFs.

Table 3. Fermentation Yield values and standard deviation of the series expressed in gSCFAs/gVS.

	T4	T8	CHA 4	CHA 8	CLI 4	CLI 8
Yield (gSCFAs/gVS)	**0,33**	**0,40**	**0,34**	**0,40**	**0,29**	**0,34**
DS	0,018	0,018	0,014	0,015	0,017	0,009

The Y_F values remained constant with a range of 0.33-0.40 gSCFAs/gVS for the T4 and T8 series, similarly for the CHA 4 and CHA 8 series with 0.34-0.40 gSCFAs/gVS and finally with values of 0.29-0.34 gSCFAs/gVS for the CLI 4 and CLI 8 series, being lower than the other two.

These data are higher than those in other tannery studies such as in Tuci et al., 2022, in which the Y_F values of reactors maintained under thermophilic conditions (55°C) with an unpretreated sludge are 0.16-0.17 gSCFAs/gVS and 12 gSCFAs/gVS.

It can be assumed that the pre-treatment carried out resulted in greater solubilization of the solids and their conversion to SCFAs in greater quantities than with a non-pretreated sludge. The presence of a diverse and resilient microbial community within the reactor is critical to the success of the anaerobic digestion process and the subsequent production of SCAFs (Bhattarai *et al.*, 2023). The shown results confirm the presence of a robust microorganism community in this sludge. Moreover, zeolites may have acted as a support for the growth of adhered biomass, favoring the growth of the microorganisms.

Furthermore, the Y_F of tanning sludge was higher than that of municipal sewage sludge or the primary/sewage sludge mixture. For example in the study by Morgan-Sagastume et al. 2015, the use of sewage sludge for PHA production by implementing anaerobic digestion under thermophilic conditions resulted in Y_F values of 0.27±0.02 gSCFAs/gVS. These are lower than those obtained in this work.

Hence, tannery sludge can be used for this anaerobic digestion process for the production of SCFAs as the results look promising for further continuous trials and future expansion of the technology.

4.5 SCFAs Composition

In Figure 14, the proportions of short-chain fatty acids (SCFAs) are illustrated, represented as the ratio between the concentration of a specific acid and the total concentration of SCFAs (in percentage, $gCOD_{ACID}/gCOD_{SCFA}$).

The T4 and T8 series exhibit similarities in the abundances of valeric acid and isobutyric acid (1% COD_{val}/COD_{SCFA} and 2% COD_{But}/COD_{SCFA}). The abundances of butyric acid are very similar in T4 and T8 (15% and 16% COD_{But}/COD_{SCFA}), as well as those of isovaleric acid (4% and 3% COD_{Isov}/COD_{SCFA}). The most noticeable difference concerns acetic acid, which is more prevalent in T8 (57% COD_{Ac}/COD_{SCFA}) compared to T4 (47% COD_{Ac}/COD_{SCFA}). In contrast, propionic acid constitutes 31% of COD_{Prop}/COD_{SCFA} in T4 and 21% in T8.

Valeric, isobutyric, butyric, and isovaleric acids are present in similar quantities in the CHA 4 and CHA 8 series. The main difference lies again in acetic acid, which is the most abundant (44% and 54% COD_{Ac}/COD_{SCFA}), unlike propionic acid (24% and 18% COD_{Prop}/COD_{SCFA}).

In the CLI 4 and CLI 8 series, acetic acid remains the most abundant but in lower percentages (37% and 36% COD_{Ac}/COD_{SCFA}) compared to the other series. Isovaleric acid is twice as abundant in CLI 4 (10% COD_{Isov}/COD_{SCFA}) compared to CLI 8 (4% COD_{Isov}/COD_{SCFA}). Isobutyric acid (3% and 2% COD_{Isob}/COD_{SCFA}), butyric acid (19% and 20% COD_{But}/COD_{SCFA}), and propionic acid (29% and 34% COD_{Prop}/COD_{SCFA}) are present in similar abundances, the latter being higher than in CHA 4 and CHA 8.

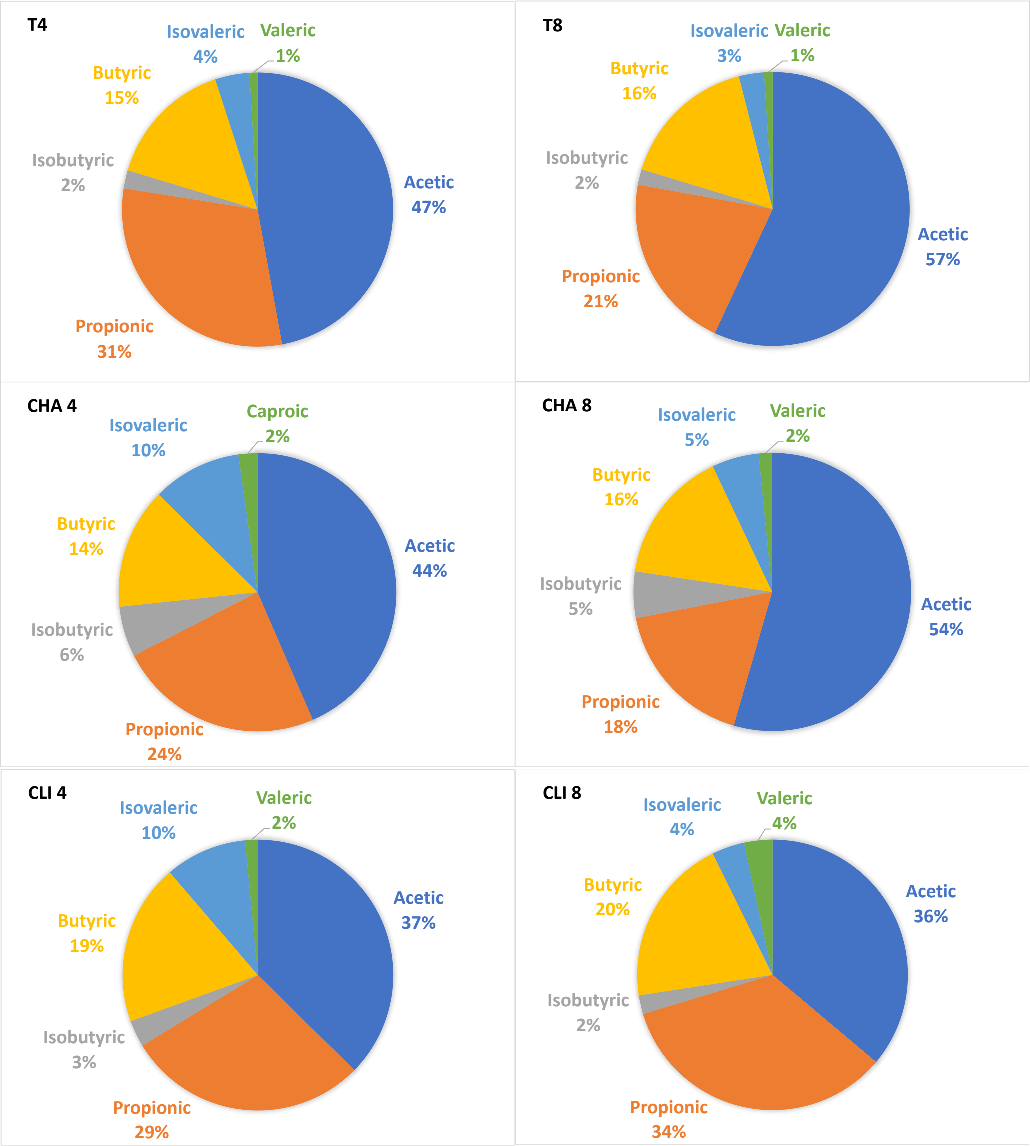


Figure 14. Composition in terms of %COD of SCFAs obtained from the thermophilic T4, T8, CHA 4, CHA 8, CLI 4, and CLI 8 tests.

In comparison to the acidogenic fermentation of the OFMSW-WAS mixture (Valentino *et al.*, 2019) the main SCFAs are partially different in this study. In the analysis of Valentino et al. 2019, butyric acid constituted 41-42% (based on COD), whereas in our study butyric acid is present in lower amounts, being the third most abundant acid (T4 15%, T8 16%, CHA4 14%, CHA8 16%, CLI4 19%, and CLI8 20%). Acetic acid was present at 22-23% (based on COD), while in our study it was the most abundant acid compared to the others (47%, 57%, 44%, 54%, 37%, and 36%).

Propionic acid (11-10%) was the third most abundant in the study by Valentino et al. 2019, whereas in our study it is the second most abundant acid, ranging from 34% to 18%. Valeric acid, on the other hand, was present in non-negligible quantities (12-11%) in the study by Valentino and colleagues in 2019, whereas it is very low in our results. The predominance of butyric acid over acetic acid in the study by Valentino et al. 2019 is probably attributable to the use of OFMSW (Gottardo *et al.*, 2017).

In the study by Lorini et al. 2022 the main acids observed during thermally pretreated WAS fermentation were acetic, butyric, and propionic acid, with percentages of 55-22-18%, respectively, and in the fermentation of thermally pretreated WAS they were 58-19-15%. Also in this research, acetic, butyric, and propionic acid were the most abundant, but in different proportions.

The results obtained show the predominance of acetic, butyric, and propionic acid although in different percentages in the three studies, thus showing a similarity between WAS and tannery sludge in the final composition of SCFAs.

It should be noted that, in the work of Strazzera and colleagues in 2021, the composition of SCFAs appears to be different from this study. In particular, butyric acid emerges as the predominant acid, accounting for 50% of the total weight, followed by valeric, acetic, and propionic acids, which constituted 14%, 7%, and 6.5%, respectively.

The differences in the composition of SCFAs can be attributed to various factors, such as the structures of the two zeolites, which may have influenced the growth of bacterial communities capable of producing the different acids, selecting those mainly responsible for the production of acetic and propionic acid. In addition, the type of substrate may also have influenced the selection of certain microorganisms with a metabolism suited to growing in sludge with a high protein component and also composed of other chemicals from the tanning process and other waste products.

4.6 Behavior of soluble chromium in the presence of natural zeolites

Figure 15 shows the chromium concentrations measured in the three experimental runs. The limit of quantification for total chromium is 0.1 mg/L and for Cr(VI) is 0.03 mg/L. The values refer exclusively to trivalent chromium, as this was the only species detected in the samples.

Following the thermal pretreatment implemented, the initial chromium concentration detected in the liquid fraction was 355 mg/L.

Chromium and heavy metals in general are usually found bound to the organic substance and the use of a pre-treatment, in this case thermal, allowed for its release in the liquid fraction (Appels *et al.*, 2010). This process accounts for the initial high concentration of dissolved chromium in the effluent. This value highlights the disparity between the thermophilic pre-treatment conducted in this study and that reported in the study by Tuci et al. 2023. In the latter, the chromium concentration after mild oxidizing pre-treatment of the sludge under mesophilic conditions was 119±11 mg/L. It can be inferred that thermal pre-treatment and thermophilic conditions have a greater capacity to solubilize chromium (III) than the H_2O_2 pretreatment and mesophilic conditions.

The trend shows a high concentration on the first day, followed by a gradual decrease in all series. The difference between the minimum concentrations is evident when comparing the series without zeolite with those using chabazite and clinoptilolite. The T4 and T8 series can be regarded as equivalent both in terms of measured concentrations and the decrease trend. It can be said that the different hydraulic retention times (HRT) played no role in the diversification of the two series. In both runs, a decrease in concentration is observed at day 23, reaching values of 133 mg/L for T4 and 153 mg/L for T8, then maintaining approximately constant concentrations until the end of the experiments.

In contrast, in the zeolite-amended runs, the chromium concentrations decreased over time to an undetectable zero concentration in the sample. In contrast to the T4 and T8 runs, the two CHA 4 and CLI 4 runs with shorter retention times reached zero before the CHA 8 and CLI 8 runs, for the former two at 28 days and 39 and 42 days for the latter two.

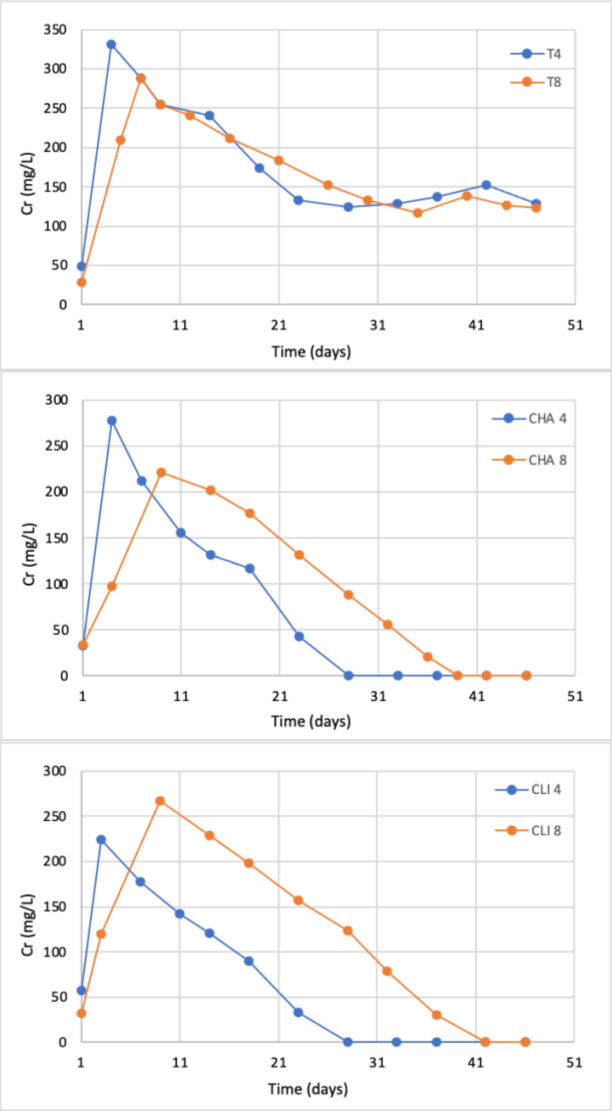


Figure 15. Trends of Cr concentrations (mg/L) in T4, T8 CHA 4, CHA 8 CLI 4, and CLI 8.

Thus, the use of zeolites had a 100% efficiency on the removal of trivalent chromium, compared to about 60% removal for the series without zeolites.

It could already be assumed that the T4 and T8 series performed differently from the others due to the absence of zeolites. The reduction in Cr(III) concentrations can be attributed to a change in its speciation, with precipitation in the form of $Cr(OH)_3$ under neutral pH conditions, favored by the presence of OH^- ions from the lime already present in the sludge as also explained by Tuci et al. 2023. The pH also plays a key role in this process; indeed, a study by Wang and colleagues in 2018 showed decreases in chromium concentrations under slightly alkaline pH conditions.

These mechanisms may explain how some fractions of Cr^{3+} are removed, but they are not effective enough to completely remove it, as can be seen in the T4 and T8 series.

In conclusion, to obtain a 100% removal of the large quantities of chromium present in the effluent, it is necessary to resort to the use of zeolites.

The results confirm that both zeolites are highly effective tools for the adsorption of dissolved trivalent chromium and its removal from the dissolved phase. The absence of this toxic element makes the safe and worry-free use of the effluent for the extraction of fermentation-derived SCFAs possible. It should be emphasized that this study is not an isolated case, as significant chromium removal efficiency of zeolites is found in other research contexts. The work of Barra-Hinojosa et al. 2024 involving the use of Neonite zeolite also showed the removal of 97% of total chromium, acting under different operating conditions.

4.7 Behavior of ammonia in the presence of natural zeolites

The ammonia concentrations measured during the reactor stability period are stable. Figure 16 shows a steady trend in this parameter without any significant changes in concentration in the different series.

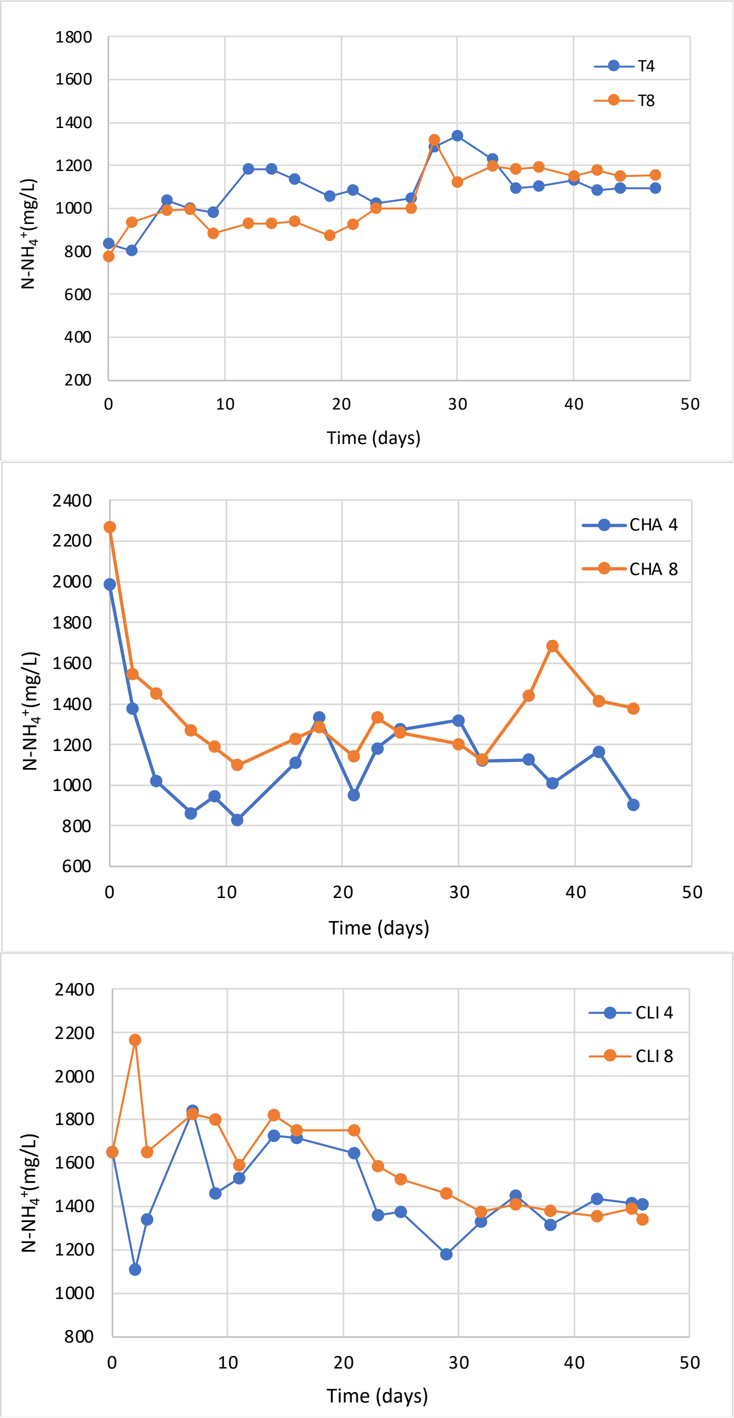


Figure 16. Trends of NH_4^+ concentrations (mg/L) in T4, T8 CHA 4, CHA 8 CLI 4, and CLI 8.

Considering the average values measured over the stability period shown in Table 4, a similarity is evident between the values of the tests performed without zeolite (T4 and T8) and the tests treated with chabazite (CHA 4 AND CHA 8). The values of the series treated with clinoptilolite (CLI 4 and CLI 8) are significantly higher, which is also associated with a higher amount of release (63 and 64%).

Table 4. Ammonia nitrogen and released nitrogen values and their standard deviations.

	T4	T8	CHA 4	CHA 8	CLI 4	CLI 8
N-NH_4^+ (mg/L)	**1127**	**1078**	**1098**	**1291**	**1478**	**1518**
DS	23	33	45	45	47	47
N-NH_4^+ release (mgN-NH_4^+/mgTKN)	**48%**	**46%**	**47%**	**55%**	**63%**	**64%**
DS	1%	1%	2%	2%	2%	2%

Since the ammonia concentration remained constant without any decrease, it can be said that it was not absorbed by the zeolites. Despite its presence, the fermentation process was not inhibited, allowing the continued production of volatile fatty acids.

The fact that the ammonia concentrations are higher in the presence of clinoptilolite can be justified by the evidence found in Tang and colleagues in 2023 in which it is shown that the use of natural zeolites in activated sludge leads to an increase in the hydrolysis efficiency of organic matter and ammonia, comparable to the trend found in this study.

On the contrary, however, it has not been demonstrated that zeolite can reduce ammoniacal nitrogen in water through ion exchange and adsorption capacity as shown by Lin et al. 2013 and Montalvo et al. 2012. More precisely, the mechanism of clinoptilolite's selectivity for ammonium is delineated through the cationic exchange between Na^+ ions, which initially occupy the zeolite's extra-framework exchange sites, and NH_4^+ from the surrounding environment, as attested by Valdés, Pérez-Cordoves, and Díaz-García 2006.

In this sludge, the failure to absorb ammonia can be attributed to the abundant presence of Na^+ ions, deriving from the use of sodium-containing salts during the tanning process, as sodium chloride (NaCl) is one of the most common chemicals in the tanning industry, mainly used in tanning, soaking, and pickling processes (Li *et al.*, 2016).

Moreover, this process might be impacted also by the presence of other dissolved substances in the sludge in ionic form such as the calcium ion (Ca^{2+}) used in the liming process. The calcium ion has a similar ionic radius to the sodium ion and can be assumed to have interacted with the zeolite, negating the possibility of ion exchange between Na^+ ions and NH_4^+. The observed significative absorption of dissolved trivalent chromium may also have interacted with the zeolites, denying ammonia access to the adsorbent sites.

5 Conclusion

This study aims to assess the possibility of using tannery sludge for the production of Short-Chain Fatty Acids (SCFAs) under thermophilic conditions, implementing a thermal pretreatment and employing semi-continuous Continuous Stirred Tank Reactors (CSTR). A novel aspect introduced compared to other similar studies is the use of natural zeolites to specifically reduce concentrations of dissolved chromium and ammonia.

The highest SCFAs production was observed in the series without zeolite with the longest retention time, reaching a maximum value of 22.1 g/L with an acidification yield of 44%. Conversely, for the series treated with chabazite and an 8-day retention time, the maximum recorded value was 21.7 g/L, similar to the previous one, showing little difference in SCFAs production between the series without zeolite and the one with chabazite. The difference is more noticeable in the series treated with clinoptilolite, which showed lower values compared to other series with longer residence times, reaching a maximum value of 18.3 g/L. Overall, the results obtained are positive, with similar growth trends in all series.

In this study, it was found that tannery sludge acts as a pH regulator, maintaining a neutral value of around 7 due to the presence of lime deriving from tanning processes and this characteristic was further enhanced by the presence of zeolites acting as a buffer. In series using zeolites, pH levels remained remarkably stable over time, exhibiting smaller variations compared to series not treated with zeolites. This characteristic is significant since, in similar processes, acidification caused by the accumulation of organic acids typically decreases the pH, requiring the use of chemicals to maintain the optimal pH conditions for microorganisms, which was not necessary in this specific case.

Promising values of Chemical Oxygen Demand (CODsol) conversion to volatile fatty acids were obtained, especially in tests where chabazite was added, improving efficiency up to 92% for the SCFA/CODsol ratio. Considering other series, values ranged between 71% and 75%, still positive. The composition of SCFAs differed in all series, with a prevalence of acetic, propionic, and butyric acids, which are the shorter-chain acids. The variation in composition could be attributed to the presence or absence of zeolites and the two different types of zeolites used, potentially influencing the growth and specific selection of certain bacterial species. Additionally, the type of substrate proved to be a key factor in the development of specific microorganisms compared to other types of sludge.

Conversely, no significant differences were detected in fermentative yield values, with the lowest values recorded in the series with clinoptilolite.

The most evident aspect is that zeolites showed a remarkable ability to absorb chromium from the liquid phase up to the point of zero concentration. The removal of this toxic element allows for safe and worry-free extraction of SCFAs from the effluent produced during fermentation. In contrast, in tests without zeolites, the reduction was 60%, with an average residual dissolved chromium concentration calculated between days 23 and 21 of 134±10 and 132±12 mg/L for T4 and T8, respectively.

Regarding ammonium ion removal, the hypothesis of zeolites removing it through ion exchange was not confirmed. Concentrations of this ion did not decrease and remained relatively consistent across all series. However, a higher release of ammonium ion was observed in series treated with clinoptilolite.

This study is part of a broader research project aimed at better understanding this potential resource. Utilizing this substrate to obtain a renewable source of carbon is a significant step towards implementing the concept of circular economy, aligning with the European Green Deal to become the first climate-neutral continent by 2050. The potential use of these wastes offers both an environmentally sustainable solution and the reuse of sludge considered hazardous to the environment and challenging to dispose of.

In addition to the need to outline further procedures to maximize the potential of this waste, the focus should be on the economic and technical feasibility of this process, integrating it into existing tannery wastewater treatment plants.

From this work, it is evident that zeolites are effective in removing heavy metals present in the dissolved phase of these sludges, paving the way for new usage and experimentation perspectives, even on larger process scales. Besides the need to delve into the effectiveness of these natural silicates, it is crucial to conduct further research on microbial dynamics and their interaction between the solid and liquid phases to fully exploit this resource.

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