

Targeted bio-based volatile fatty acid (VFA) production from waste streams through anaerobic fermentation: link between process parameters and operating scale

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ABSTRACT

Anaerobic processes are proven to have much more environmental and economic benefits than conventional aerobic treatment systems, offering sustainable energy and valuable biochemicals. In recent years, bio-based volatile fatty acid (VFA) production has into prominence as more value is derived before ending up to other final products. This paper presents a critical review of the research studies on bio-based volatile fatty acid (VFA) production from different waste streams (i.e. industrial sludge/waste, organic fraction of municipal solid waste, municipal wastewater/sludge, combined streams) through anaerobic fermentation. Fundamentals and decisive process parameters (i.e. pH, temperature, retention time, organic loading rate) are reviewed and their correlations with VFA yields are critically discussed based on n. 178 cases (156 lab- and 22 pilot-scale). The picture we provided clearly demonstrated that process parameters should be clearly defined and optimized according to the type of waste streams which may have a significant impact on downstream processes in most cases.

KEYWORDS: *bio-based; fermentation; process parameters; resource recovery; sewage sludge; volatile fatty acid*

INTRODUCTION

Establishing solid interconnections between the existing value chains and creating new ones are the fundamentals towards the transition to bio-based circular economy. This consolidation can further lead to more circular and cost-effective industrial processes and new job opportunities. Biorefinery concept is an important contributor to a sustainable bioeconomy to fulfil the renewable energy targets of European Union (EU) as 20% by 2020 and 32% by 2030 ¹. Biorefineries can play a crucial role to maintain sustainable resource recovery and management ². In a biorefinery concept, the extraction and production of high added-value products through innovative technologies have greater priority than bioenergy production ^{3,4}. The so-called “waste” materials, such as agricultural

waste, sewage sludge, municipal waste, are important substrates in many biotechnological processes for sustainable production.

In the last years, enormous efforts have been given by the water industry and scientific community to develop new approaches that can recover resources from water while meeting the water quality standards⁵. In this period, next generation wastewater treatment plants (WWTPs) have shifted towards water resource recovery facilities (WRRFs) and become an important case of biorefineries as they use innovative processes for bio-based production of new chemicals and materials from carbon-rich waste streams⁶. The bio-based production of volatile fatty acids (VFAs) fits well within this concept as they represent valuable sources for downstream processes such as electricity generation by microbial fuel cells, biodiesel production by oleaginous yeast, hydrogen production via photofermentation, and synthesis of other valuable commodity chemicals⁷. VFAs serve as starting molecules for bioenergy production and for the synthesis of a variety of products, such as bioplastics^{3,8}. In fact, the value of these products, e.g. polyhydroxyalkanoate (PHA), a precursor for bioplastics, can be up to 4 times higher than that of methane ($\text{€}1.2 \text{ kg}^{-1} \text{ COD}_{\text{PHA}}$ vs. $\text{€}0.14\text{--}0.26 \text{ kg}^{-1} \text{ COD}_{\text{CH}_4}$)⁹. A three-step approach, “capture-ferment-upgrade”, has been commonly proposed and followed for this scheme that comprises the capture of chemical oxygen demand (COD) as sludge, its conversion to VFAs and subsequent upgrading into valuable products⁹. Currently, most practices that target VFAs production typically utilise food waste/organic fraction of municipal solid waste (OFMSW) and sewage sludge^{3,10}, while other waste flows should not be overlooked due to their promising potential such as industrial waste/wastewater with relatively high organic content (e.g. cheese whey permeate, pulp and paper mill effluents)¹¹. However, the link between process parameters and efficiency of these processes were still not taken together in a comprehensive paper, which represents the main motivation of this paper.

BIO-BASED VFA PRODUCTION THROUGH ANAEROBIC FERMENTATION

Fundamentals

VFAs are comprising groups of aliphatic monocarboxylic acids with chain lengths of up to seven C atoms, namely formic (C1/HFo), acetic (C2/HAc), propionic (C3/HPr), iso-butyric (iC4/iHBu), n-butyric (C4/HBu), iso-valeric (iC5/iHVa), n-valeric (C5/HVa), iso-caproic (iC6/HCa), n-caproic (iC6/HCa) and n-enanthic (C7/HEn) acids ¹². VFAs are valuable substrates to a variety of applications. In the wastewater sector, VFAs can be used to support and increase the removal of nutrients (nitrogen and phosphorus) in biological processes such conventional activated sludge (CAS) and enhanced biological phosphorus removal (EBPR) ^{13,14}. Alternatively, VFAs are advantageous for the production of biogas, biodiesel, bioplastics, and biohydrogen as well as electricity via microbial fuel cells ⁶.

VFAs production is traditionally based on non-renewable petrochemical sources, causing serious negative health and environmental effects ⁶. Given the numerous applications, more sustainable methods are getting attention to produce VFAs. Processes such as anaerobic digestion (AD) and fermentation, represent a feasible, sustainable carbon neutral method of production of VFAs. AD and fermentation can be carried on several materials used as substrates, deriving of various sources, including waste ¹⁵ Two methods are mainly used for the anaerobic production of VFA from waste: i) attached growth biomass and ii) suspended growth biomass ¹⁴. Among the attached growth systems, packed bed reactor is common, while suspended growth systems include reactors like upflow anaerobic sludge blanket (UASB) and continuous stirred-tank reactor (CSTR). In particular, the latter are the most used, as, if designed and managed correctly, they are ideal for mixing waste and microbial cultures, even in presence of high concentrations of suspended solids (SS). In some cases, the reactor is also followed by a gravity separation unit used to recirculate the biomass inside the CSTR, thus increasing the sludge retention time (SRT). Regardless of the reactor configuration, given the long retention times of the fermentation reactions, the feeds of these units are often of the semi-continuous type or with sequential charges ¹⁴.

Lab- and pilot-scale cases

The further sections of the paper under the VFA topic presents the findings of bio-based VFA production through anaerobic fermentation based on the data obtained from a total of 178 cases. The individual case studies were distinguished based on the type of waste stream and the size of the fermentation reactor, defining laboratory-scale tests those carried out in a reactor with a volume of less than 10 liters (156 cases) and pilot-scale tests (22 cases) those with larger volumes.

Waste streams for the biological production of VFAs

A variety of waste materials can be used as substrate to produce VFA, such as waste activated sludge (WAS) generated in WWTPs, food waste (FW) or OFMSW, or industrial wastewater rich in biodegradable organic matter¹⁶. VFA yield is mainly linked to the operating conditions¹⁷, and the decision about which type of waste is most suitable to produce VFA is not possible due to the different operating conditions adopted and the different performance evaluation criteria. However, it is possible to identify which parameters, or combinations of parameters are utmost critical for the fermentation process and for increasing production yields. The matrices commonly used for the fermentation process are characterized by a COD content greater than 4000 mgCOD/l. The ammonium content; on the other hand, although is necessary for the growth of biomass, should be lower than 5000 mg/l to avoid the inhibition of VFA production¹⁴. A summary of the main characteristics of the matrices and of the evidence of their application to produce VFAs is shown in

Table 1.

Table 1. Substrate characteristics for bio-based VFA production.

Matrix	Specification	Total COD (mg/L)	Soluble COD / Total COD	Evident presence of inhibitory compounds for the hydrolysis phase	Need for specific pre-treatments	Reference
Industrial sludge or waste	Dairy industries wastewater	4590-12000	0.3-0.7	-	n.d.*	11,18,19
	Paper mill wastewater	2410-26300		Contaminants from bleaching processes	YES	11,20
	Olive oil mill wastewater	28500-70400		Polyphenols	YES	21
	Palm oil mill wastewater	88000		-	n.d.	14
	Wood industries waste	11110		Inhibitory compounds	YES	22,23

Food waste	-	91900-472000	0.15-0.5	Lignocellulosic materials, fats and proteins. Residual metals	YES	24-26
Sewage sludge	Cellulosic sludge, primary sludge, secondary activated sludge, mixed sludge	14800-23000	0.01-0.1	Extracellular polymeric substances	Preferable	27,28

*n.d: not defined

Industrial bio-based streams (industrial sludge or waste)

The matrices associated with industrial processes and mostly used for fermentation are wastewater generated by agriculture, dairy, oil, wood and paper industries. Whey permeate from the dairy industry and wastewater from paper mills were found to be suitable for VFA production, as they have a high rapidly biodegradable organic content ¹¹. The characterization of paper mill wastewater was done both in two cases which reported the COD concentration as 26,300 mg/l²⁰ and 2410-7740 mg/l¹¹. In numerous case studies, the effluent of oil mills were tested. In particular, the streams obtained from palm oil production contain high concentrations of COD (on average 88,000 mg/l) and can lead to a production of VFA with concentrations up to 15,300 mg/l ¹⁴. The effluents from the olive oil mills also have COD ranging between 28,500 and 70,400 mg/l, reaching final concentrations of VFA in the range of 7,100-15,600 mg/l ^{21,29}. When oil mills wastewater is used, pre-treatments may be necessary for the removal and recovery of polyphenols. Those, indeed, in addition to be an exploitable material, can also inhibit the metabolic activity of the bacteria that produce PHAs ²⁹. Finally, several studies have also considered the production of VFA from molasses of sugar cane ³⁰ or effluents from sugar beet processing ³¹. Even the effluents generated by wood processing have been considered by some authors, highlighting their different characterization with respect to the effluents of paper mills. Wastewater from the paper industry, in fact, contains pollutants associated with bleaching processes that are not found in the effluents of wood processing ^{22,23}. Such streams are characterized by high COD contents, low pH and low nutrient concentration; however, the nature of the organic material may not be easily degraded in a

fermentation process. In particular, 35% of the total COD is comprised of hardly biodegradable material²². Furthermore, the presence of inhibitory compounds may prevent the acidogenic process²³. Consequently, the use of this type of matrix usually requires a pre-treatment step.

Organic Fraction of Municipal Solid Waste and Food Waste

Municipal organic waste is produced in large quantities (39% of municipal waste on a European scale), Food and Agriculture Organization (FAO) estimated that one third of world food production is lost or wasted along the food supply chain including the final steps like households, restaurants, and canteens²⁶. The main FW components are carbohydrates, proteins, and lipids³². Both OFMSW and FW must undergo a treatment process before disposal in order to stabilize the material to reduce its environmental impact³³. Anaerobic bio-fermentation processes can be used to produce VFAs and other low molecular weight organic compounds such as alcohols or lactic acid^{34,35}. The use of these substrates to produce VFA is favoured by the high organic content in terms of COD of about 91,900-472,000 mg/l²⁴⁻²⁶. With reference to OFMSW, it must be considered that, when the quality of the source separation is poor, it is necessary to apply specific treatments that allow to separate the organic fraction from other materials that could be present in the waste, such as glass, plastic and ferrous materials. Further pre-treatments may be necessary to optimize the hydrolysis phase which represents the limiting step in the fermentation process. Moreover, the presence of lignocellulosic materials, fats and proteins in the OFMSW lowers the rate of biodegradability and makes the hydrolysis phase the limiting step³⁶. In case of FW fermentation, the pre-treatment step is still necessary to prepare the substrate to microorganisms' activity, reducing substrate size, extracting smaller and simpler chemical compounds to improve the fermentation stage and removing inert material not suitable for biological processes²⁶.

Wastewater and sewage sludge

One of the most used matrices to produce VFA is the sludge generated by the wastewater treatment plants. Two main types of sludge are produced, with different characteristics of biodegradability and organic content: i) Primary sludge (PS) usually contains a large quantity of biodegradable

organic compounds such proteins, carbohydrates, cellulose and other organic materials ³⁷; ii) secondary sludge is made up of polysaccharide and protein rich bacteria and micro-organisms that form extracellular polymeric substances (EPS) ³⁸. Both these types of sludge, as well as their possible mixtures, can produce VFA when subjected to anaerobic fermentation. Primary and activated sludge are rich in organic matter, with COD ranging from 14,800 to 23,000 mg/l. However, the soluble COD of the sludge is normally 10 to 100 times lower than the total COD, which makes the hydrolysis process more limiting and slows down the VFA production ¹⁴. Several studies have highlighted the importance of controlling parameters such as pH, temperature and SRT in order to increase the rate of hydrolysis ^{37,39}. The co-fermentation of different types of sludge was also advantageous, allowing an increase in the production of VFA from 85 mgCOD/gVSS with only primary sludge to 118 mgCOD/gVSS by combining it with activated sludge ¹⁴. Furthermore, some studies have tested the use of primary cellulosic sludge to produce VFA. The cellulosic sludge is separated using a dynamic rotary filter (RBF) that allows to obtain a concentrated and fiber-rich matrix mainly originated from toilet paper in the wastewater ^{40,41} and, through the control of parameters such as temperature, pH and HRT, VFA production can be maximized ^{37,40}.

Combined matrices (food waste + sewage sludge)

The combination of different types of waste has been tested in several cases in order to maximize the production yields of VFA. Different studies addressed the co-fermentation of FW and sewage sludge. These two substrate have a different composition being the FW mainly composed of carbohydrates and the sewage sludge of proteins⁴². However, co-digestion of food waste and excess sludge is especially attractive since it can dilute potential toxic compounds, improve the balance of nutrients and produce synergistic effects on microorganisms, which would lead to enhanced biogas production and biogas yields⁴³. High VFA production yield has been obtained, reaching up to 0.69 gCOD/gVS with an HRT of 6 days⁴⁴. Furthermore, the combination of industrial wastewater rich in starch and sewage sludge were tested by several authors ^{45,46}, highlighting the possibility of reaching high VFA production up to 45 mgVFA/gVSS·d.

Fermentation process and process parameters

The production of VFA is accomplished through a fermentation process which involves three main stages (**Figure 1**). (i) hydrolysis: Breakdown of complex organic compounds (carbohydrates, proteins and lipids) into simpler monomers (monosaccharides, amino acids and short-chain fatty acids) (ii) acidogenesis: Fermentation of monomers (monosaccharides, amino acids) and production of intermediate VFAs (mainly acetate, propionate and butyrate) (iii) acetogenesis: Fermentation of other monomers (short-chain fatty acids) and production of acetic acid (72%) and hydrogen (28%)⁴⁷.

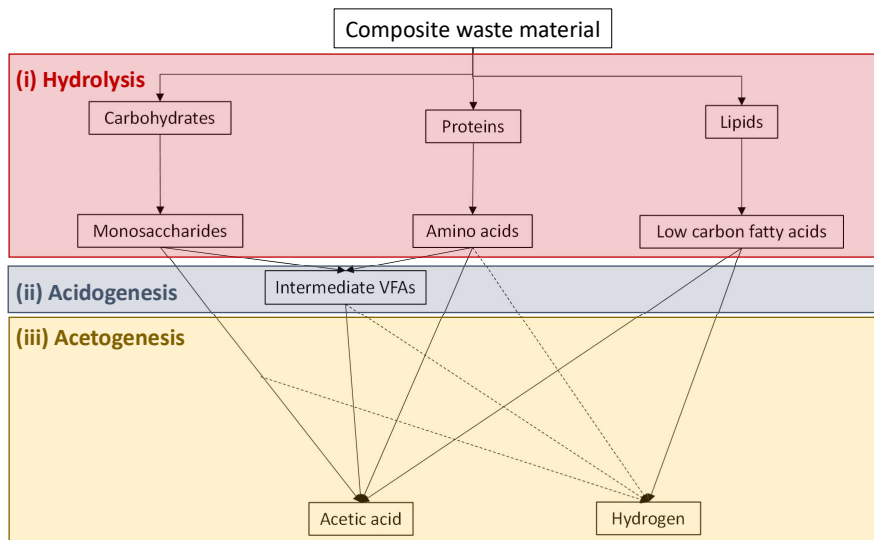


Figure 1. Three main stages of bio-based VFA production through anaerobic fermentation following three stages (i) hydrolysis (ii) acidogenesis (iii) acetogenesis.

The limiting step is often the hydrolysis³⁷. In order to increase the hydrolysis rate, it is possible to act in two distinct ways: (i) monitoring and control of key parameters (ii) application of specific pre-treatments.

Critical parameters of the fermentation process

In most cases the fermentation process is influenced by operating parameters such as pH, temperature, hydraulic retention time (HRT), solids retention time (SRT) and organic loading rate (OLR).

pH affects the metabolic activities of microorganisms. Most enzymes cannot tolerate extreme environments, both acidic (pH <3) and alkaline (pH > 12). Optimal values are generally between 5 and 11¹⁴. At the lab-scale (**Fig. 2a** and **Fig. 3a**), there are variations based on the matrix used:

- With industrial wastes, the pH is usually between 6 and 7. In most cases, this parameter is controlled through the dosage of appropriate reagents.
- For the OFMSW/FW, the pH is often controlled and usually is imposed between 5 and 6. On the other hand, in the co-fermentation processes of FW and municipal sludge, pH ranges between 5 and 6 even without the control.
- In municipal sludge, a greater variability is observed, associated with a greater number of tests performed. Except for cellulosic sludge, most of the experiments are carried out with pH control testing a wide range of values between 3 and 11.

At the pilot-scale (**Fig. 4a**), it was noted that in most cases (17 out of 22), pH control was not conducted; however, the values do not show any high variations as they are established between a minimum of 4.5 and a maximum of 7.3.

Temperature mainly influences the growth of microorganisms, the activity of enzymes and the rate of hydrolysis during the process of fermentation. Acidogenic fermentation can be carried out at different temperature ranges, based on the types of microorganisms selected: (i) mesophilic (between 35 °C and 45 °C) (ii) thermophilic (between 45 °C and 75 °C) (iii) hyperthermophilic (above 75 °C)⁴⁸.

The mesophilic condition (35 °C) is considered the most favourable from an economic point of view even if it provides a production of VFA 10 times lower than the thermophilic condition⁴⁹.

Thermophilic fermentation, moreover, exhibited advantages of higher substrate degradation rate, higher hydrogen (H₂) production, lower gas solubility of hydrogen and methane, pathogen-free effluents and efficient heat utilization for hot wastewater treatment⁵⁰. At the lab scale (**Figure 2b** and **Figure 3b**), it was observed that the fermentation of the OFMSW/FW is mainly performed at temperatures between 35 and 40 °C. For industrial wastes, the temperature is usually between 25

and 35 °C, with few cases at lower (20 °C) or higher (55 °C) temperatures. A greater variability is observed for municipal sludge with several cases at room temperature (20 °C), but also at temperature higher than 55-60 °C. Meanwhile at the pilot scale (**Figure 4b**), 90% of the cases operated at temperatures above 30 °C, in particular between 30 and 50 °C, with an average value of 43 °C.

HRT affects the substrate and its rate of hydrolysis, while SRT influences the activity of microorganisms. Generally, a longer HRT leads to a higher VFA production as the microorganisms have more time to react with the waste. Differently, lower SRT is beneficial to the production of VFA because it can prevent the dominance of methanogens in the anaerobic reactor⁵¹. In CSTR configurations without recirculation, HRT is equal to SRT, therefore the substrate and the biomass remain inside the reactor for the same time.

For the fermentation of industrial matrices, as well as of OFMSW/FW, high HRT are advantageous to produce VFAs. However, excessive increases lead to insignificant improvements, and are also economically less sustainable, as larger volumes are needed^{14, 52} reports that the degree of acidification of dairy wastewater improved from 28.2% to 54.1% increasing the HRT from 4 to 12 h. Bringing the HRT to 16 and 24 h only increased acidification slightly, to 55.8 and 59.1%, respectively. Similarly, the yield in the production of VFA from food waste increased changing the HRT from 4 to 8 days. However, no significant difference between 8 and 12 days was observed³³. In laboratory tests (**Figure 2c** and **Figure 3c**) with industrial-type matrices, the HRTs were very low and, only in one case, exceed two days. The fermentation of the OFMSW/FW occurs at HRT comprised between 3.5 and 8 days. Great variability is observed also for municipal sludge, with values that fluctuated, in most cases, between 5 and 8 days. Lower HRTs were adopted for primary sludge (4.2 ± 1.9 days), while the highest values for WAS (9 ± 4.6 days). The greatest variability; however, is more evident in the combined matrices in which retention times vary between a minimum of 1 and a maximum of 9 days. In the cases at a pilot scale (**Figure 4c**), the retention times were quite constant even between the different matrices and almost all comprised between 6

and 8 days. There is only one case with a higher HRT (equal to 14 days) for the fermentation of municipal sludge and two cases with a lower HRT (equal to about 2 days) for the fermentation of OFMSW/FW.

As already mentioned, lower SRT is beneficial to the production of VFA nevertheless, the SRT should be sufficiently long to promote hydrolysis of the sludge ¹⁴. There is a very limited number of studies on the influence of SRT on the VFA production. Only for 17 cases out of the 178 reviewed, the value of this parameter was provided. SRT is generally found between 7 and 15 days, except for industrial sludge which has lower values (2.6 ± 0.3 d).

OLR represents the organic mass load fed daily per unit of fermentation volume. Based on the cases analysed, we could not determine any clear influence of the OLR on VFA production. Most commonly, the organic load of the fermentation process ranged from 4 to 25 gCOD/L/d. The fermentation of industrial matrices (liquid waste and/or sludge), OFMSW/FW and primary cellulosic sludge generally operated at organic loads between 9 and 12.7 gCOD/L/d with average values of 9.2 ± 7.5 , 9.9 ± 3.3 and 11 ± 5.4 gCOD/L/d, respectively. For the sewage sludge fermentation, only in one case, an OLR of 20-30 gCOD_{sol}/L/d was applied ⁵³.

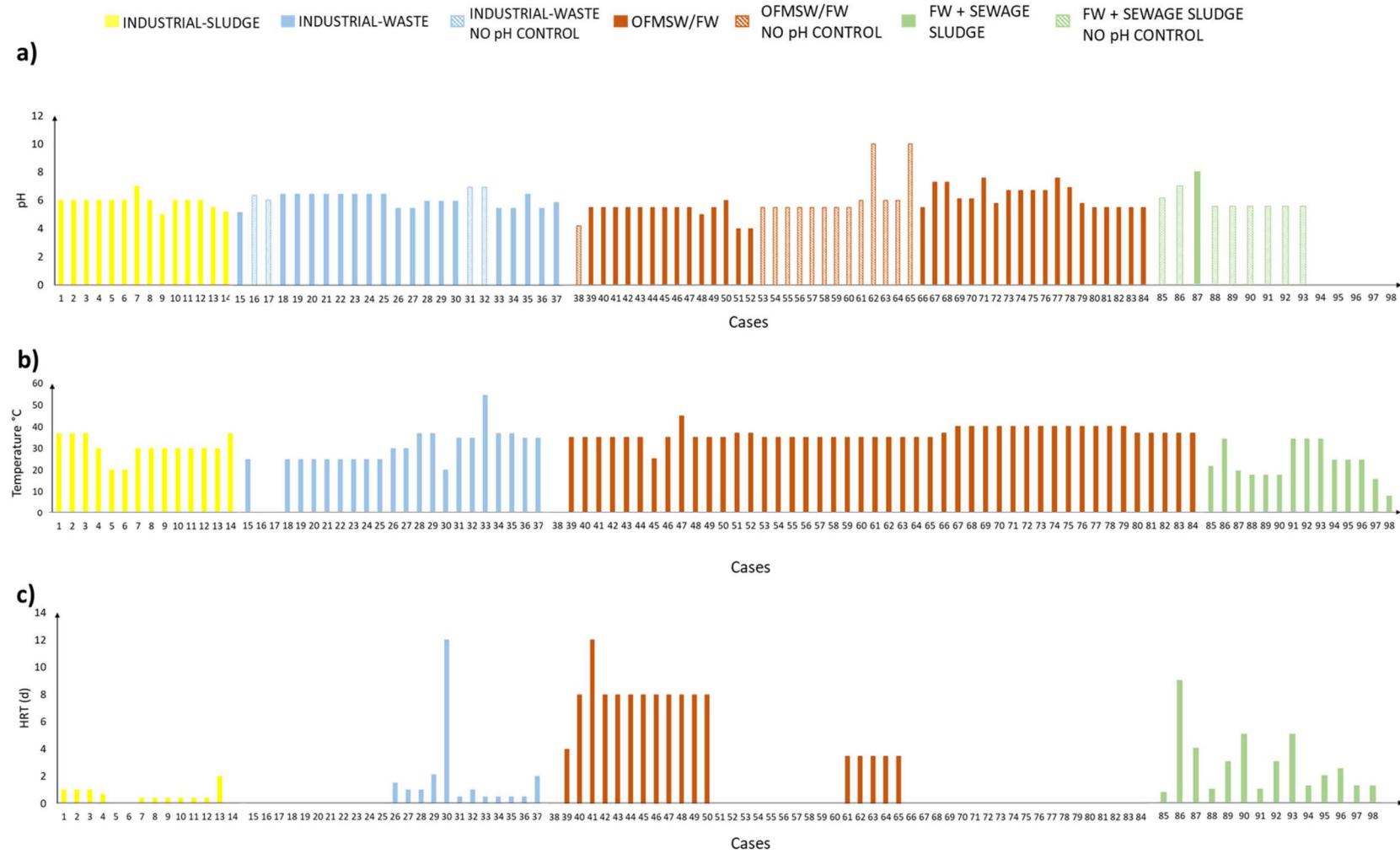


Figure 2. Different conditions of a) pH b) temperature c) HRT during the fermentation of bio-based streams at lab-scale (part 1).

Case 1-3⁵⁴, 4⁵⁵, 5-6²⁰, 7-10³⁰, 11⁵⁶, 12⁵⁷, 13⁵⁸, 14⁵⁹, 15²⁹, 16-17⁶⁰, 18-25²¹, 26²², 27²³, 28-29¹¹, 30²⁰, 31-32¹⁸, 33-35⁶¹, 36⁶², 37³¹, 38⁶³, 39-50³³, 51-52⁶⁴, 53-60⁶⁵, 61-65⁶⁶, 66²⁴, 67-79⁶⁷, 80-84²⁴, 85⁴⁶, 86⁴³, 87⁴², 88-93⁶⁸, 94-98⁴⁵

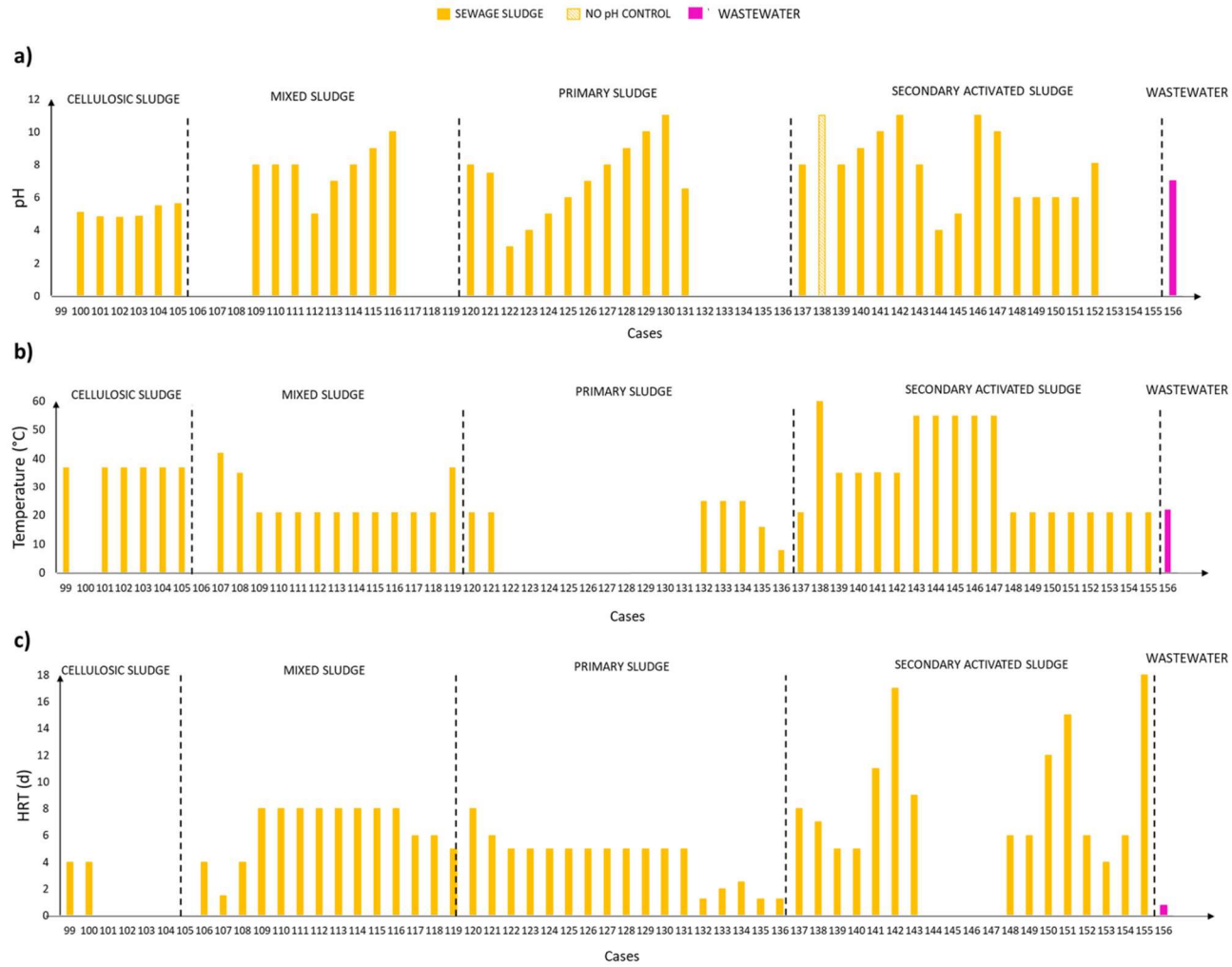


Figure 3. Different conditions of a) pH b) Temperature c) HRT during the fermentation of bio-based streams at lab-scale (part 2). Case 99-100³⁷, 101-105⁴⁰, 106⁶⁹, 107⁵³, 108⁷⁰, 109-116⁷¹, 117-118²⁸, 119⁷², 120⁷¹, 121²⁸, 122-131⁷³, 132-136⁴⁵, 137⁷¹, 138⁷⁴, 139-147⁷⁵, 148-151⁷⁶, 152-155²⁷, 156⁴⁶

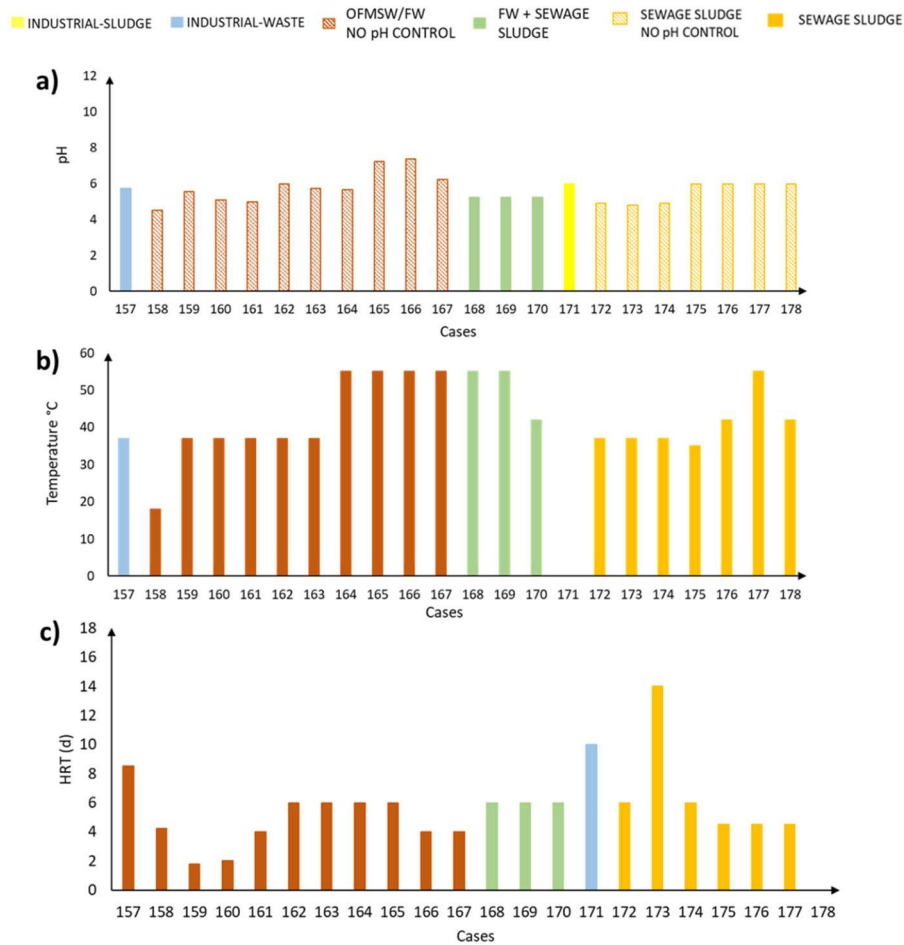


Figure 4. Different conditions of a) pH b) Temperature c) HRT during the fermentation of bio-based streams at pilot-scale. Case 157⁵⁹, 158³⁵, 159-163⁷⁷, 164-167⁷⁸, 168-170⁷⁹, 171⁸⁰, 172-174⁴⁰, 175-177⁸¹, 178⁸²

Fig. 5 shows the correlations between the analysed parameters based on average values in the cases both at laboratory- and pilot-scale. At the lab-scale (**Fig. 5a**), it is noted for the OFMSW/FW that the fermentation process for the production of VFA operates at an average temperature of around 37° C, with HRT of about 6 days, and an organic load equal to 10 gCOD/L/d. The pH, in this case and for all the other matrices, is between 5 and 7. Very similar values of temperature and HRT also characterize the fermentation of sewage sludge. On the other hand, OLR is applied higher for this matrix (25 gCOD/L/d). Finally, the fermentation of industrial waste occurs at lower HRT (2-3 d) and at the OLR of 10-12 gCOD/L/d and temperature of 25-30 °C. In the pilot case studies (**Figure 5b**), for all the matrix types considered, the average pH values are again between 5 and 7 and the

HRTs between 5 and 10 days. The process temperature, on the other hand, is much more variable and probably associated with evaluations of the energy sustainability of the entire process and on average equal to i) 50 °C in the case of the combination of sewage sludge and FW, ii) 40 °C in the case of sewage sludge and ii) 35 °C for industrial waste.

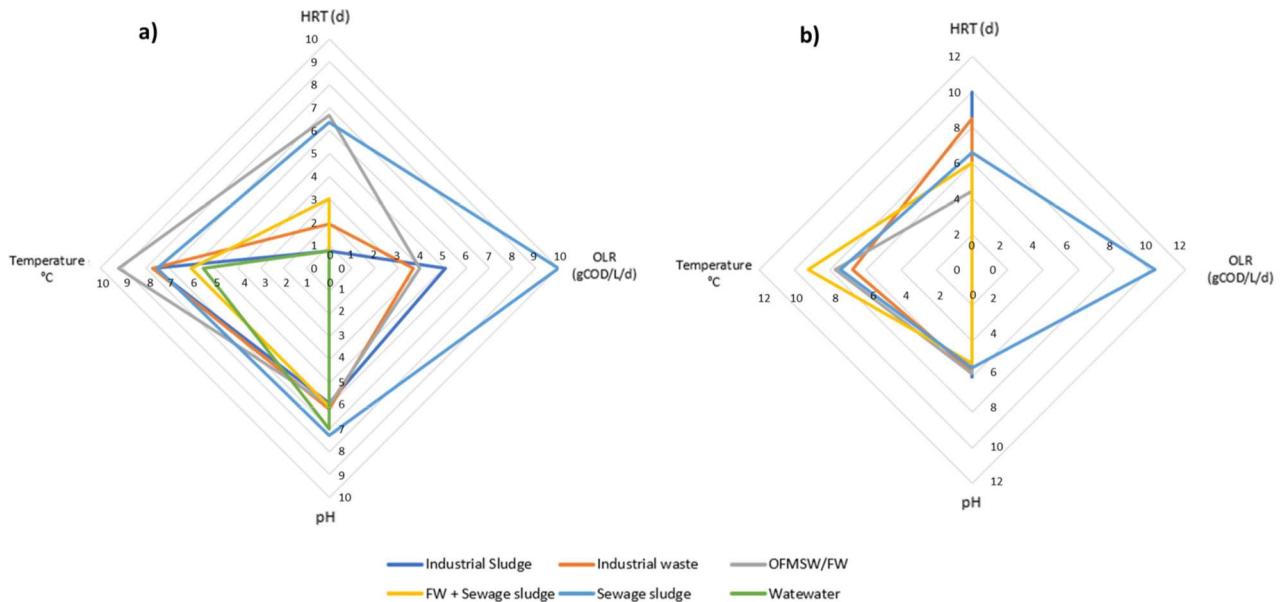


Figure 5. Correlation between the critical parameters for a) lab-scale and b) pilot-scale.

Pre-treatment conditions

A pre-treatment may be necessary not only to increase the rate of hydrolysis, but also to optimize the biological process to produce VFAs from waste streams. Physical, chemical, biological and thermal processes can be applied both individually and in combination. Chemical treatment usually involves the application of reagents, such as acids, alkalis, ozone and hydrogen peroxide¹⁴. The use of acids is more effective in removing microorganisms that consume hydrogen and, by repressing methanogenic activity, protects the bacteria from spores²⁴. The use of alkali reagents also suppresses the growth of hydrogen-consuming microorganisms²⁴. However, the use of acids requires corrosion-resistant equipment due to the extreme pH values that are reached. Ozone can also be used, acting as a strong oxidizing agent that causes the splitting of hydrogen bonds, as well as swelling and decomposition of biomass components⁸³. A further pre-treatment adopted to improve the solubilization of solid waste involves the use of biological agents such as hydrolytic

enzymes. Several commercial enzymes have been tested for the hydrolysis of FW, while by dosing a mixture of enzymes, better results were obtained than single type of enzymes. This is because different enzymes hydrolyze different components of the waste ⁸⁴. Among the physical pre-treatments, both microwave irradiation and ultrasound treatments are applied. Microwave irradiation is electromagnetic radiation with a wavelength between 1 mm and 1 m, corresponding to an oscillation with a frequency of 0.3-300 GHz ⁸⁵. It is applied for both sewage sludge and OFMSW for breaking hydrogen bonds and destabilizing the structure of molecules. It can lead to the formation of recalcitrant compounds that decrease the biodegradability of the treated solid waste ¹⁴. Ultrasound treatment, on the other hand, causes a localized pressure to drop below the evaporation pressure with the formation of micro-bubbles. The vacuum, created by the collapse of these micro-bubbles, determines the production of mechanical forces capable of eroding the solid particles ⁸⁶. Alternatively, a thermal pre-treatment can be applied. Thermal pretreatment has been studied using a wide range of temperatures ranging from 60 to 270 °C. In practice, the optimum temperature is in range of 160-180°C and treatment times from 30 to 60min ⁸⁷The thermal shock is effective in removing the microorganisms that consume hydrogen, protecting the bacteria responsible for the formation of spores, in this way, methanogenic activity is also inhibited ²⁴. The application of high temperatures, in addition to a high energy consumption, may lead to the formation of recalcitrant soluble organic substances. On the contrary, the pre-treatments at temperatures below 70 °C involves the production of bacteria that improve the solubilization of the material ¹⁴.

Achieved bio-based VFA production

The production yield of VFA represents the degradation capacity of the complex organic substances into simple monomers and is expressed in grams of COD (as VFA) per grams of COD (as VSS) (gCOD(VFA)/gCOD(VS)). Moreover, the productivity is a specific parameter that represents the production capacity of VFA with respect to the unit of time and the volume of the reactor and is expressed in gCOD(VFA)/L/d.

Yields and productivity

The lab-scale case studies showed that the maximum production yields (gCOD(VFA)/gCOD(VS)) was achieved through the fermentation of the OFMSW/FW and the cellulosic-type sewage sludge, with values of 0.45 ± 0.11 gCOD/gCOD and 0.5 ± 0.17 gCOD/gCOD, respectively. In terms of productivity (gCOD(VFA)/L/d), higher results of 2.2 ± 0.78 gCOD(VFA)/L/d are observed again for the OFMSW/FW. Significantly lower values were obtained for primary and secondary sewage sludge (0.5 and 0.27 gCOD(VFA)/L/d, respectively). For the pilot scale case studies, a smaller number of applications were found, but no high variability is observed. In particular, the combination of FW and municipal sludge showed a yield equal to 0.5 ± 0.13 gCOD(VFA)/gCOD(VS). Furthermore, FW and its combination with municipal sludge also represented the matrices with the highest productivity, with values equal to 4.1 ± 2.6 gCOD(VFA)/L/d and 3.3 ± 0.3 gCOD(VFA)/L/d, respectively. Finally, in terms of correlation and influence between the process parameters and the production yields, it is possible to mention that the highest yields were obtained at the temperature between 25 °C and 45 °C, HRT of less than 10 days and pH between 5 and 7 (**Figure 6**).

Finally, a summary of the principal parameters analysed in the most important case studies is given in **Table 2**.

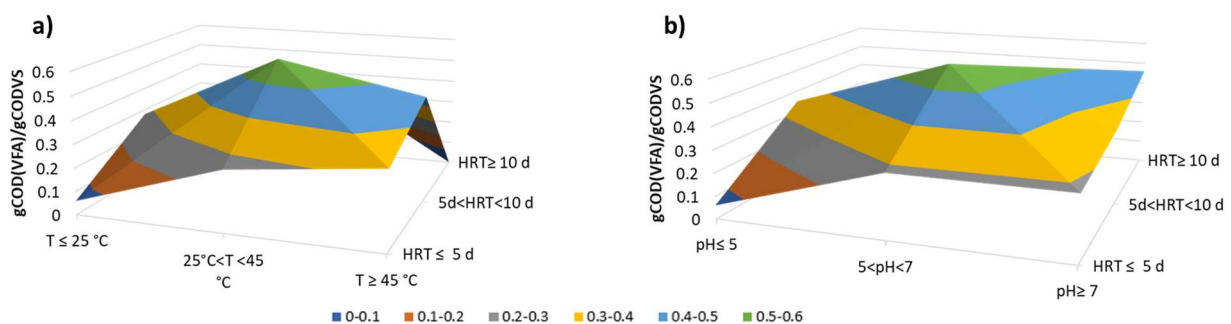


Figure 6. Changes in VFA yields in correlation with the main process parameters (a) temperature and HRT (b) pH and HRT.

Table 2. Summary of principal characteristics in the main case studies analyzed.

Source of wastewater	Pre-treatment	COD concentrations (mg/l)	VFA concentration (gCODVFA/l)	VFA Yield (gCOD(VFA)/gCODVS)	Reference
Lab scale					
Cheese whey (CW)	No pre-treatment		9.09		
Sugar cane molasses (SCM)	No pre-treatment		12.37		54
Paper mill effluent	Nutrient addition		6.07		55
Paper mill effluent	Sparged with nitrogen gas to remove any dissolved oxygen		13.38		20
Olive oil mill effluent	No pre-treatment	36900	10.03		29
Olive oil mill effluent	Solid phase extraction		13.27		60
Olive oil mill effluent	No pre-treatment	28500	6.66		
Olive oil mill effluent	Addition of Bentonite	28500	6.84		
Olive oil mill effluent	Addition of Bentonite and centrifugation	28500	11.62		
Olive oil mill effluent	No pre-treatment	70400	14.62		
Olive oil mill effluent	Addition of Bentonite	70400	14.53		
Olive oil mill effluent	No pre-treatment	36700	8.62		
Olive oil mill effluent	Addition of Bentonite and centrifugation	36700	11.25		
Olive oil mill effluent	Centrifugation	36700	12.19		21
Wood mill effluent	No pre-treatment	11110	1.31		22
Wood mill effluent	No pre-treatment	11110	1.87		23
Paper mill effluent	Nutrient addition	7740	2.13		
Cheese whey	Nutrient addition	4590	3.71		11
Dairy wastewater (OLR = 9.3 gCOD/l/d)	Nutrient addition	4420	1.41		
Dairy wastewater (OLR = 4.2 gCOD/l/d)	Nutrient addition	4420	1.03		18
Dairy wastewater	Nutrient addition	4000	1.50		61
Dairy wastewater	purged with nitrogen to remove any dissolved oxygen	12000			19

Gelatin-rich proteinaceous wastewater	No pre-treatment	4000	1.41	0.17	88
Pharmaceutical wastewater	No pre-treatment	50000	3.28	0.17	62
Sugar industry wastewater + pressed beet pulp	Wastewater was settled for 1-h to remove the inorganic suspended materials. Beet-pulp was thawed at room temperature and further dried at 105 °C for 24 h. Then, the dried pulp particles were grinded by the help of a pestle.	6621	3.37		31
Mixed food waste (boiled rice, cooked vegetables, un-cooked vegetables (spoiled), cooking oil, vegetable peelings, cooked meat, boiled spices)	FW was masticated using electrical blender and filtered through stainless steel sieve to remove coarse materials that cause clogging. The oil fraction present in the FW was separated through gravity separator		3.75		63
Food wastes collected from a cafeteria (HRT = 4d; T = 35°C; pH = 5.5)	No pre-treatment		5.16		
Food wastes collected from a cafeteria (HRT = 8d; T = 35°C; pH = 5.5)	No pre-treatment		12.66		
Food wastes collected from a cafeteria (HRT = 12d; T = 35°C; pH = 5.5)	No pre-treatment		19.92		
Food wastes collected from a cafeteria (HRT = 8d; T = 35°C; pH = 5.5; OLR = 5 gCOD/l/d)	No pre-treatment		12.66		
Food wastes collected from a cafeteria (HRT = 8d; T = 35°C; pH = 5.5; OLR = 13 gCOD/l/d)	No pre-treatment		27.61		
Food wastes collected from a cafeteria (HRT = 8d; T = 25°C; pH = 5.5; OLR = 9 gCOD/l/d)	No pre-treatment		15.94		
Food wastes collected from a cafeteria (HRT = 8d; T = 35°C; pH = 5.5; OLR = 9 gCOD/l/d)	No pre-treatment		22.03		33

Food wastes collected from a cafeteria (HRT = 8d; T = 45°C; pH = 5.5; OLR = 9 gCOD/l/d)	No pre-treatment		18.56	
Food wastes collected from a cafeteria (HRT = 8d; T = 35°C; pH = 5; OLR = 9 gCOD/l/d)	No pre-treatment		7.12	
Food wastes collected from a cafeteria (HRT = 8d; T = 35°C; pH = 6; OLR = 9 gCOD/l/d)	No pre-treatment		23.44	
Potato solids (500 g)	No pre-treatment		20.14	
Potato solids (1000 g)	No pre-treatment		12.73	64
OFMSW from MBT (pH = 6)	Pre-treatment at the MBT plant to remove certain undesired materials + particle size reduction, adjustment of the water content of the feedstock to the wet AD process, and removal of precipitable inerts and floating materials		15.71	
OFMSW from MBT (pH = 10)			16.51	
FW collected from a university canteen (pH = 6)	Blended with deionized water and shredded		14.32	
FW collected from a university canteen (pH = 10)	Blended with deionized water and shredded		8.08	66
FW from Dufferin Organics Processing Facility (DOPF)	No pre-treatment	91900	8.39	24
Cattail (17.8 gVS/l; pH = 7.3)	No pre-treatment		0.46	
Cattail (6.5 gVS/l; pH = 7.3)	No pre-treatment		0.49	
Cattail (17.8 gVS/l; pH = 6.1)	No pre-treatment		0.38	
Cattail (6.5 gVS/l; pH = 6.1)	No pre-treatment		0.45	
Cattail (12.2 gVS/l; pH = 7.6)	No pre-treatment		0.46	
Cattail (12.2 gVS/l; pH = 5.8)	No pre-treatment		0.17	
Cattail (20.3 gVS/l; pH = 6.7)	No pre-treatment		0.43	
Cattail (4.1 gVS/l; pH = 6.7)	No pre-treatment		0.59	
Cattail (12.3 gVS/l; pH = 6.7)	No pre-treatment		0.51	
Cattail (12.3 gVS/l; pH = 6.7)	No pre-treatment		0.52	67

Cattail (12.3 gVS/l; pH = 7.6)	No pre-treatment		4.08	
Cattail (4.1 gVS/l; pH = 6.9)	No pre-treatment		1.74	
Cattail (5.8 gVS/l; pH = 5.8)	No pre-treatment		1.63	
FW from Dufferin Organics Processing Facility (DOPF)	Ultrasonic		12.28	
FW from Dufferin Organics Processing Facility (DOPF)	Heat shock		11.62	
FW from Dufferin Organics Processing Facility (DOPF)	Acid		11.06	
FW from Dufferin Organics Processing Facility (DOPF)	Base		9.07	
FW from Dufferin Organics Processing Facility (DOPF)	Ultrasonic + acid		15.84	24
Mixture of primary sludge and industrial wastewater (Old Dutch Food Company)	No pre-treatment		0.54	46
Artificial food waste (88%) + municipal sludge (12%)	No pre-treatment		27.19	43
WAS + FW (from a cafeteria)	Food waste was crushed by an electrical blender and diluted to 25% (v/v)	22125	7.87	42
Kitchen food waste (from a cafeteria) (25%) + primary sludge (75%) (HRT = 1d; T = 18°C)	FW was blended after diluting it four times using tap water		3.37	
Kitchen food waste (from a cafeteria) (25%) + primary sludge (75%) (HRT = 3d; T = 18°C)	FW was blended after diluting it four times using tap water		0.94	
Kitchen food waste (from a cafeteria) (25%) + primary sludge (75%) (HRT = 5d; T = 18°C)	FW was blended after diluting it four times using tap water		0.23	
Kitchen food waste (from a cafeteria) (25%) + primary sludge (75%) (HRT = 1d; T = 35°C)	FW was blended after diluting it four times using tap water		2.34	
Kitchen food waste (from a cafeteria) (25%) + primary sludge (75%) (HRT = 3d; T = 35°C)	FW was blended after diluting it four times using tap water		0.34	68

Kitchen food waste (from a cafeteria) (25%) + primary sludge (75%) (HRT = 5d; T = 35°C)	FW was blended after diluting it four times using tap water		0.15		
Cellulosic primary sludge (CPS) (pH = 8)	No pre-treatment		12.47	0.57	
Cellulosic primary sludge (CPS) (pH = 9)	No pre-treatment		16.59	0.76	
Cellulosic primary sludge (CPS) (pH = 10)	No pre-treatment		11.26	0.51	
Cellulosic primary sludge (CPS) (pH = 11)	No pre-treatment		7.50	0.34	40
PS/WAS (volatile suspended solids (VSS)-based)=25/75 (pH = 8)	Settling 24 h at 4°C			0.23	
PS/WAS (volatile suspended solids (VSS)-based)=50/50 (pH = 8)	Settling 24 h at 4°C			0.36	
PS/WAS (volatile suspended solids (VSS)-based)=75/25 (pH = 5)	Settling 24 h at 4°C			0.24	
PS/WAS (volatile suspended solids (VSS)-based)=75/25 (pH = 7)	Settling 24 h at 4°C			0.36	
PS/WAS (volatile suspended solids (VSS)-based)=75/25 (pH = 8)	Settling 24 h at 4°C			0.40	
PS/WAS (volatile suspended solids (VSS)-based)=75/25 (pH = 9)	Settling 24 h at 4°C			0.39	
PS/WAS (volatile suspended solids (VSS)-based)=75/25 (pH = 10)	Settling 24 h at 4°C			0.30	
PS/WAS (volatile suspended solids (VSS)-based)=100/0 (pH = 10)	Settling 24 h at 4°C			0.36	
PS/WAS (volatile suspended solids (VSS)-based)=0/100 (pH = 8)				0.09	71
Primary sludge + WAS	No pre-treatment	22256		0.17	
Primary sludge + WAS + SDBS (0.02 g/g)	No pre-treatment	22949		0.25	
Primary sludge	No pre-treatment	22838		0.12	28
Primary sludge + WAS	No pre-treatment	15480	0.08	0.41	72
Primary sludge (pH = 3)	Sludge was concentrated by settling at 4 °C for 24 h	20631	0.91		
Primary sludge (pH = 4)			1.59		73

Primary sludge (pH = 5)			2.25	
Primary sludge (pH = 6)			2.38	
Primary sludge (pH = 7)			2.94	
Primary sludge (pH = 8)			3.15	
Primary sludge (pH = 9)			3.18	
Primary sludge (pH = 10)			3.29	0.44
Primary sludge (pH = 11)			3.42	
Activated sludge	Samples were concentrated by settling at 4 °C for 24 h		2.40	0.38
Activated sludge (HRT = 5d; pH = 8; T = 35°C)				74
Activated sludge (HRT = 5d; pH = 9; T = 35°C)				0.37
Activated sludge (HRT = 5d; pH = 9; T = 35°C)				0.43
Activated sludge (HRT = 11d; pH = 10; T = 35°C)				0.46
Activated sludge (HRT = 17d; pH = 11; T = 35°C)	Sludge was concentrated by settling at 4 C for 24 h	18657		0.46
Activated sludge (HRT = 9d; pH = 8; T = 55°C)				0.53
Activated sludge (pH = 4; T = 55°C)				0.06
Activated sludge (pH = 5; T = 55°C)				0.19
Activated sludge (pH = 10; T = 55°C)				0.05
Activated sludge (pH = 11; T = 55°C)				0.29
Activated sludge			0.18	75
Activated sludge + SDS (0.05 g/g)	Sludge was concentrated by settling at 4 °C for 24 h	14890	0.94	
Activated sludge + SDS (0.1 g/g)			2.10	
Activated sludge + SDS (0.25 g/g)			2.87	27
Municipal Wastewater	No pre-treatment		0.51	46
Pilot scale				
Organic municipal solid wastes from source-sorting collection	Removal of metals, drum sieve (trommel) for the removal of plastics and cardboard or wood pieces before a second shredding in a 5 mm cutter			
			0.06	35

Organic fraction of municipal solid waste (HRT = 1.8d)	No pre-treatment		18.30		
Organic fraction of municipal solid waste (HRT = 2d)	No pre-treatment		11.04		
Organic fraction of municipal solid waste (HRT = 4d)	No pre-treatment		17.12		
Organic fraction of municipal solid waste (HRT = 6d)	No pre-treatment		12.19		77
Organic fraction of municipal solid waste (HRT = 6d)	No pre-treatment		18.36		
Organic fraction of municipal solid waste (HRT = 4d)	No pre-treatment		15.97		78
OFMSW + thickened sewage sludge (T = 55°C)	Squeezing and homogenization of OFMSW		19.69	0.57	
OFMSW + thickened sewage sludge (T = 42°C)	Squeezing and homogenization of OFMSW		17.81	0.58	79
Cellulosic primary sludge (CPS) (OLR = 17.7 kgVS/m ³ d)	No pre-treatment		7.84	0.22	
Cellulosic primary sludge (CPS) (OLR = 5.7 kgVS/m ³ d)	No pre-treatment		7.83	0.20	
Cellulosic primary sludge (CPS) (OLR = 7.5 kgVS/m ³ d)	No pre-treatment		9.35	0.47	40
Activated sludge (T =35 °C)	No pre-treatment	43000	5.62	0.30	
Activated sludge (T =42 °C)	No pre-treatment	43000	7.69	0.39	
Activated sludge (T =55 °C)	No pre-treatment	43000	6.66	0.39	81
Activated sludge	No pre-treatment		6.56	0.39	82

Influence of process parameters on the composition of VFA

This section discusses the influence of process parameters on the composition of the VFAs. In particular, it should be noted that both temperature and retention time do not have a great influence on the type of VFA as much as pH does¹⁴.⁸⁹ showed that acetic acid is produced at the highest levels in acidic conditions ($44 \pm 7\%$); a mixture of acetic acid ($35 \pm 8\%$) and butyric acid ($37 \pm 4\%$) is dominant in neutral conditions, while butyric acid ($60 \pm 12\%$) is dominant in alkaline conditions (pH 8 - 10). This was also previously reported by⁹⁰ who highlighted that acetic acid was the dominant VFA at pH 5.5, while butyric acid at pH 8-10. In another study⁶⁶; however, the highest percentage of acetic acid was achieved at pH 9 and 10, with 60.5% and 60.2%, respectively. This is consistent with the findings of²⁵, who also observed that a pH between pH 9 and 11 favored the production of acetic acid in the treatment of organic waste. At neutral pH (pH 6 and pH 7.5), approximately 15% butyric acid and 20% caproic acid were produced. Finally, the study by⁷¹ reported that acetic and propionic acids were the two most common short chain fatty acids (SCFAs) in all pH conditions. The acetic acid content decreased from 47% to 28% with increasing pH from 5.0 to 8.0, while it increased to 55.8% when the pH reaches the value of 10. The propionic acid fraction increased from 20% to 45% as the pH increased in the range of 5-8, which further decreased to 21% when the pH increased to 10. The other two SCFAs produced simultaneously were the butyric acid fraction which decreased from 21% to 12% by changing the conditions of pH from 5 to 10, and the content of valeric acid remained relatively constant.

Finally, the influence of OLR on VFA composition was analyzed⁹¹ and reported that the increase of OLR (from 5 to 12 kgCOD/m³/d) changed the predominant VFA type from acetic acid to butyric acid. The increase of butyric acid with OLR was also reported by⁹² which found out that at a medium OLR of 10 g COD/L/d, propionic acid was the second main acid, but it was substituted by butyric acid at a higher OLR of 26 g COD/L/d. On the other side, the reduction of acetic acid with an increased OLR was reported by⁹³ for the fermentation of synthetic dairy wastewater: as the OLR increased from 4 gCOD/L/d to 24 gCOD/L/d, the percentage of acetate declined from 53% to 22%,

whereas the propionate percentage rose from 13% to 41% under mesophilic condition. Similar trend was observed in the thermophilic operation whereby the percentages of acetate and propionate changed from 44% to 23%, and from 21% to 43%, respectively ¹⁴.

VFA products: Types and economic valorisation alternatives

The main type of VFAs are acetic acid, butyric acid, propionic acid and caproic acid. VFAs can be either directly used in a WWTP or recovered to provide products to the market. The recovery of VFAs from waste streams is a crucial process to obtain value-added products. Atasoy et al.⁶ reviewed VFA recovery processes from waste streams which include gas stripping with absorption, ion exchange, electrodialysis, solvent extraction, nanofiltration, reverse osmosis and membrane reactors. The recovery process will eventually bring higher total costs for VFA production from waste streams. For instance, reverse osmosis or high voltage electrodialysis can be costly due to high energy cost, while they may recover VFA with higher purity. In that sense, recovery processes should be selected carefully according to the specific application purposes.

Each VFA species can be used for numerous applications, including the production of plastics, textile finishes, pharmaceuticals, pesticides, biofuels beverages and cosmetics ^{6,94-96}. The bulk prices of the main short-chain fatty acids produced from fossil sources are shown in **Table 3**. Acetic acid is a crucial compound in the paint, textile, pulp & paper and plastic industries. Moreover, it is also used in food production as an acidity regulator and preservative. Acid producing bacteria can produce cellulose and also have a role in the beverage and food industries to produce specific foods and drinks. Propionic acid is used in the food sector in the form of sodium and/or calcium salts as a food preservative. Propionic acid is also used in various industries related to herbicide production, green solvents for coating, animal feed, and vitamin E production. Butyric acid is one of the most important sources for biodiesel production. It became attractive in the animal feeding industry due to its anti-pathogenic features. Since antibiotic usage in the animal is limited by regulations, butyric acid is used as an antibiotic and supplement in animal husbandry. Similar to propionic acid, bio-based butyric

acid is also used as a food flavoring ingredient. It also shows anti-cancer activity causing biochemical transformation in the cells⁶.

VFAs are also used to produce bioplastics or biopolymers, for instance PHAs, which are seen as the key alternative to petroleum-derived products due high biodegradation rates and green production ways. They can also serve as a biofuel or building block for the synthesis of various chemicals⁹⁷. It is important to underline that the composition of the VFAs directly influence the yield, productivity and monomer composition of the PHA. Acetate and butyrate, indeed, tend to generate 3-hydroxybutyrate (3HB) monomers, while propionate and valerate produce 3-hydroxyvalerate (3HV). The monomer composition of PHA subsequently determines the thermal and mechanical properties of the polymer, including its elasticity, crystallinity and stiffness ⁹⁸.

Table 3. Economic valorisation of VFA ^{6,94}.

VFAs	Chemical formula	Price (USD/ton)	Application
Acetic acid	CH ₃ COOH	400-800	Vinyl acetate monomer, Food additive, Solvent, Vinegar, Ester production, Chemicals
Propionic acid	CH ₃ CH ₂ COOH	1500-1700	Animal and human food additive, Chemical intermediate, Solvent, Flavouring agent
Butyric acid	CH ₃ (CH ₂) ₂ COOH	2000-2500	Esters used food industry as aroma additive, Food additive, flavoring, Pharmaceuticals, Animal feed supplement, Fishing bait additive
Caproic acid	CH ₃ (CH ₂) ₄ COOH	2000-2500	Pharmaceuticals, flavors, feed additives, antimicrobials, plant growth promoters, paint additives

VFAs have various potential applications for on-site usage in WWTPs. They can have a role in biological nutrient removal (BNR) processes in which biodegradable organic carbon is required as an electron donor and energy. In many cases, wastewater does not include sufficient amount of biodegradable organic carbon and hence organic carbon such as methanol, glucose and acetate, is supplied externally for efficient BNR⁹⁹. In this case, VFAs recovered from wastewater can act as a carbon source in biological nutrient removal units, replacing methanol or other external substrates. Denitrification is a biological process in which reduction of nitrate and nitrite to gaseous forms of nitrogen occurs. Acetate is the most preferred VFA during the denitrification since it is an easily degradable feature by many bacteria, followed by butyrate and propionate. Propionate has been

found to enhance denitrifying biological phosphorus removal via nitrite due to the increase in the amount of phosphorus-accumulating organisms¹⁰⁰.

Challenges and future aspects

The final recovery of VFA is as important as the fermentation process and optimization of VFA production⁶. Due to the complex physical-chemical nature of the fermentation broth and low concentration of acids in the system, separating VFAs from the fermentation broth is a challenging task which represents the main barrier for the scale up to industrial level¹⁰¹. The acid content of a VFA-containing aqueous solution is a critical parameter to consider for choosing a suitable VFA separation method¹⁰². Among the recovery methods mentioned earlier, membrane-based processes such as nanofiltration and water electrodialysis attract much attention as being easy and efficient methods for VFA recovery^{95,103}. Furthermore, the need to treat the effluent streams containing significant amount of VFAs has been recognized to meet current and future water quality standards. Alkali metal salts of these compounds are entirely soluble in water and insoluble in hydrocarbon media causing significant environmental pollution. Therefore, it is important to remove VFAs both for pollution control reasons but also for their use in the industry⁹⁵.

SUMMARY AND OUTLOOK

Given their high potential as renewable carbon sources, VFAs stand forward as the perfect candidate for many bio-based processes. In order to optimise and maximise downstream processes that benefit from VFAs, it is important to design and operate an efficient anaerobic fermentation process. It is not very possible to have a certain point on the type of waste that is most suitable for VFAs production since different operating conditions have a significant influence on the type and yield of VFAs. In most cases, VFAs are obtained through the fermentation of agro-alimentary waste, food waste and sewage sludge. The case studies showed that the maximum VFAs production yield is achieved by the fermentation of OFMSW/FW and/or cellulosic-type sewage sludge. Considering the waste types we analysed, neither temperature nor retention time do not have a great

influence on the composition of VFAs as much as pH does. Further research and development are still required to optimise VFA production at a large scale.

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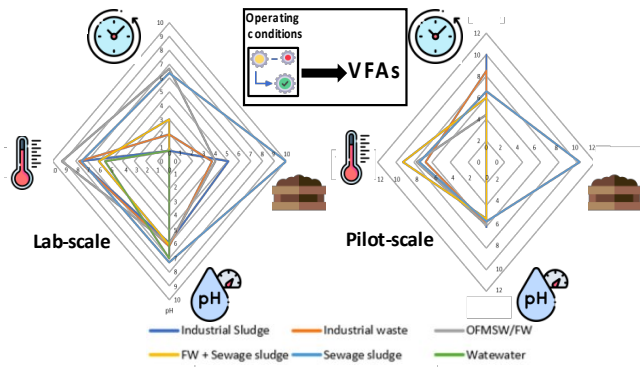
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In a sustainable bio-based VFA production system, operating parameters should be well-established based on the type of waste stream which further influences VFA yields.

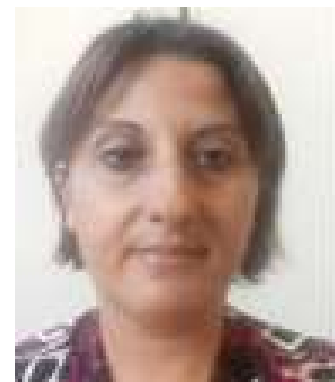
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responsibility of the Water thematic area.