Review about bioproduction of Volatile Fatty Acids from wastes and wastewaters: Influence of operating conditions and organic composition of the substrate

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ABSTRACT

Volatile fatty acids (VFAs) are a group of carboxylic acids considered as building block chemicals. Nowadays, commercial production of VFAs is performed using fossil fuel sources. As an alternative, acidogenic fermentation of wastes by mixed microbial cultures (MMC) is starting to be considered as a potential bioproduction process that would replace conventional production processes and contribute to the circular economy. Nevertheless, more research is needed to control the VFA production yields and to precisely drive the fermentation process to the production of a certain VFA or a mixture of VFAs, either by modifying the operational parameters or by appropriately tunning the substrate composition. Following this gap, this review starts screening the metabolic routes that yield VFAs by anaerobic fermentation. Subsequently, the effect of different operational parameters on VFA production yield and VFA composition distribution is extensively discussed depending on the organic composition of the waste in terms of proteins, carbohydrates and lipids. To the best of our knowledge, previous review articles analyzed the impact of these parameters for different types of wastes, but without specifically considering their organic composition in terms of proteins, carbohydrates and lipids. Afterwards, energy-based metabolic models are presented as the one of the best modelling approaches to predict VFA composition. Then, polyhydroxyalkanoates (PHAs) production by MMC is described since it is one of the most promising applications of waste derived VFAs. Finally, we highlight the research gaps that should be further investigated to develop a large scale VFA bioprocess based on MMC platform from waste streams.

1. Introduction

Volatile fatty acids (VFAs) are a subgroup of fatty acids, ranging from two to five carbon atoms, including acetic, propionic, isobutyric, butyric, iso-valeric and valeric acids. VFAs have a wide range of applications in numerous areas, thus they are considered building block chemicals. Among the VFAs, acetic, propionic, and butyric acids are those most industrially produced. The annual global market demand of acetic, propionic and butyric acids was estimated in 18.5 Mt for 2020 [1]. In food industry, acetic acid is used as vinegar as well as food additive and preservative; propionic acid is utilized in the preservation of food grains and in animal feed while butyric acid is employed as flavoring [2–5]. Other applications of acetic acid include terephthalic acid production, which is then used in the manufacture of polyethylene terephthalate (PET), and the production of acetate esters [6]. Propionic acid is also a building block in the pharmaceutical industry, and it is

employed in the production of herbicides and propionate esters for perfumes [4]. Butyric acid is widely used in the chemical industry in the production of cellulose acetate butyrate for the synthesis of thermoplastics and in the pharmaceutical industry for the manufacture of drugs with several therapeutic effects [5]. VFAs also have potential applications as renewable feedstock such as for polyhydroxyalkanoate (PHA) synthesis [7], production of electricity in microbial fuel cells (MFCs) [8], production of hydrogen [9], production of biofuels [10] or even as a source of organic matter for biological nutrient removal [11]. Finally, there are further products that can be obtained from VFAs, such as fatty alcohols, hydrocarbons, rhamnolipids, sophorolipids, N-acylethanolamines or lycopene [12,13].

Commercial production of VFAs is normally based on chemical synthesis starting from petrochemical raw sources [14]. Acetic acid is mainly obtained by methanol carbonylation, followed by other processes such as the catalytic oxidation of acetaldehyde, ethylene, or butane [15,16]. Propionic acid is traditionally produced as a by-product of acetic acid production or by the hydroxycarboxylation of ethylene in the presence of a catalyst of rhodium or nickel carbonyl [17]. Butyric acid is usually produced by oxidation of butyraldehyde, that is, synthesized by oxosynthesis of propylene obtained from crude oil. Another conventional process is the extraction of butyric acid from butter, but this method is very expensive [18]. However, due to climate change concerns and fossil fuel depletion, VFA bioproduction via microbial fermentation is gaining interest. Moreover, the final cost of the VFAs produced from petrochemical resources depends on the price of fossil fuels.

In the last years, several studies related to fermentative bioproduction of VFAs from different carbon sources have been published. Most of the VFA bioproduction by fermentation is carried out by using pure substrates, such as glucose, xylose, or glycerol [19–21]. However, even though the use of pure carbon sources should lead to high yields and productivities, the cost of this kind of substrates is high and raises the overall cost of the process. To reduce the costs for making competitive the fermentative bioproduction process compared to the fossil-based one, different types of waste, such as lignocellulosic biomass, waste activated sludge (WAS), food waste, dairy wastewater, paper mill wastewater or even olive mill wastewater have been proposed as substrates [22–28]. In addition, the use of wastes to bioproduce VFAs contributes to the implementation of a circular economy model and avoids the use of edible raw materials to produce chemicals [29]. The main disadvantage of using waste as a raw material of the fermentative bioproduction is the cost of the downstream purification process of the final products. Yet, when using pure carbon substrates, side products are not normally produced, so the purification step is inexpensive [15]. Moreover, some types of waste need pretreatment before fermentation. For example, anaerobic microorganisms are not able to directly digest solid waste, such as lignocellulosic biomass or WAS so physical, chemical or enzymatic pretreatments might be needed [30].

Hence, in the design of a VFA bioproduction process, the following items must be considered: VFA production yield, productivity, raw material costs, need of a pretreatment step and downstream processing costs [15,31]. One alternative to increase the productivity, avoid the formation of side products, and use different carbon sources is the utilization of engineered strains [12]. Nevertheless, in this case, the cost of the process will also increase due to the requirement of sterile conditions. For that reason, many researchers are focusing their work on VFA biosynthesis through anaerobic fermentation by mixed microbial cultures (MMC), which do not require sterile conditions and are considered more robust systems [32].

Thus, VFA bioproduction by fermentation of wastes using MMC is a potential and true alternative to the conventional processes based on petrochemical resources or to the biological processes based on the use of pure organic feedstocks and pure bacterial strains. Nevertheless, research in this field must overcome two challenges: (1) to understand how to drive the fermentation process to a targeted VFA or to a specific

mixture of VFAs by tuning the operational conditions or design parameters; (2) to improve the efficiency of the downstream purification methods.

Regarding the downstream purification methods for recovering VFAs like gas stripping with absorption, adsorption, solvent extraction, electrodialysis, microfiltration, reverse osmosis and nanofiltration, membrane contactor and in-line recovery have been extensively discussed in the literature [33–43] and are out the scope of this review.

In this sense, this review only covers the VFA production by anaerobic fermentation of waste streams by MMC, focusing on the first mentioned challenge. Therefore, first of all, the metabolic routes for the anaerobic production of VFAs are summed up according to a detailed literature review. Subsequently, the operational parameters affecting VFA yield and VFA composition distribution are discussed considering the organic composition of the waste streams in terms of proteins, carbohydrates and lipids. Following that, energy-based metabolic models for the prediction of VFA distribution are reviewed. After that, PHA production by MMC from waste-derived VFAs is presented to exemplify how to link the VFA bioproduction process with a real potential application. Finally, conclusions about the state-of-the-art of VFA bioproduction by MMC and research gaps are pointed out.

To the best of our knowledge, previous review articles analyzed the influence of the operational parameters on VFA production yield and VFA composition for different 'types of waste' without considering the particular organic composition the different types of wastes can have. Since these works found contradictory results, especially when analyzing the VFA composition, this review article wants to consider the waste attending to its organic composition (carbohydrate, protein and lipid content) instead of its classification as a certain 'type of waste'.

2. Metabolic routes for anaerobic production of VFAs

Volatile Fatty Acids are produced during anaerobic digestion of organic matter. Anaerobic digestion is a well-known technology that is implemented at industrial scale for biogas production [44]. Nonetheless, lately, anaerobic digestion is gaining interest in the production of other value-added products, such as VFAs and hydrogen. The anaerobic digestion process consists in the biological reduction of organic matter in absence of oxygen or nitrate/nitrite and takes place in four interdependent steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig. 1) [45]. When biological process is stopped on the acidogenesis and acetogenesis and the methanogenesis does not take place, the process is usually named anaerobic or acidogenic fermentation instead of anaerobic digestion. Hydrolysis consists in the conversion of hardly soluble compounds such as complex carbohydrates, proteins and lipids into monosaccharides, amino acids, and long-chain fatty acids. In the acidogenic step, the products of the hydrolysis are transformed by several fermentative reactions performed by facultative and obligatory anaerobes into CO_2, hydrogen, VFAs and other products, such as ethanol and lactic acid. During acetogenesis, VFAs which cannot be transformed into methane by methanogenic microorganisms, are converted into acetate, hydrogen, and CO_2. Finally, methanogenesis is carried out by two different groups of Archaea: the acetoclastic methanogens produce methane and CO_2 from acetate while the hydrogenotrophic methanogens produce methane from hydrogen and CO_2 [46,47].

Since anaerobic digestion is performed by MMC, different pathways would take place and several by-products and intermediates can be formed. Hydrolytic bacteria are strict anaerobes that include *Bacteroides*, *Clostridium*, *Micrococcus*, *Butyrivibrio*, *Selenomonas* and *Streptococcus* [46]. The acid-forming bacteria, including acetogens and homoacetogens, are responsible for VFA synthesis [48]. Acetogens can help fermentative bacteria to perform the hydrolysis step by transforming non-biodegradable organic macromolecules into smaller biodegradable molecules. After hydrolysis, acetogenic bacteria consume the obtained monosaccharides, amino acids and long-chain fatty acids and store them
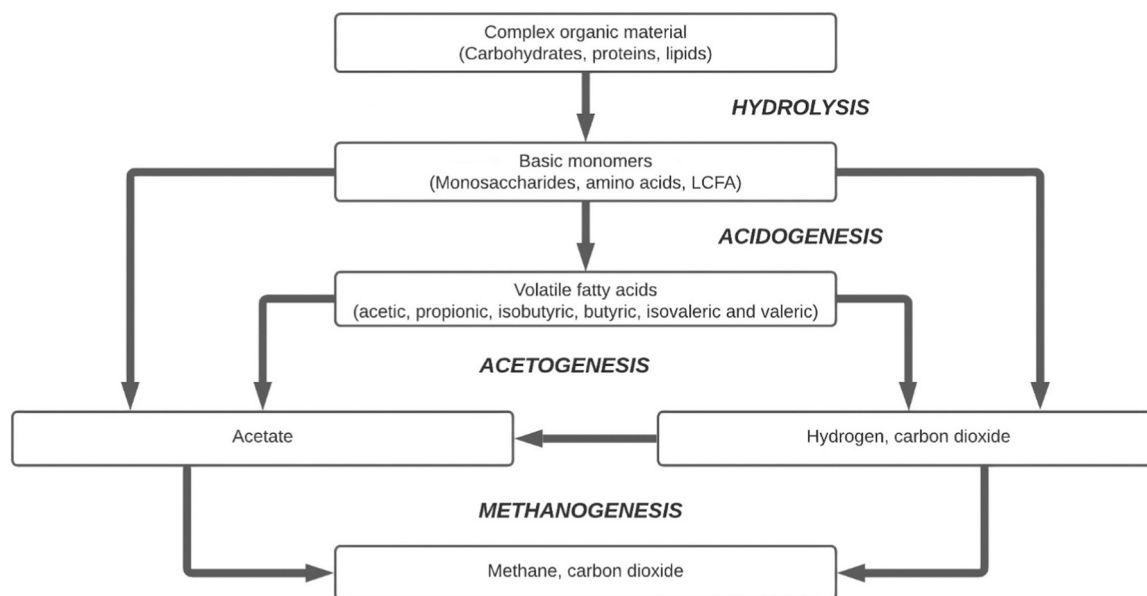
Fig. 1. Steps in Anaerobic Digestion Process.
(adapted from Ersahin et al. [59]).

intracellularly as pyruvates, which later are converted into acetate or other VFAs [49,50]. Polysaccharides are broken into monosaccharides and then, converted to pyruvate by following different pathways such as: Embden-Meyerhof pathway (EMP), Entner-Doudoroff pathway (ED), Pentose Phosphate pathway (PPP), Bifidus pathway and Phosphoketolase pathway (PK) [51,52]. Finally, pyruvate is transformed into VFAs, ethanol, lactic, H_2 and CO_2 [51]. Proteins are broken into polypeptides and amino acids. Subsequently, the latter can be converted into VFAs by three possible pathways: oxidation-reduction of pairs of amino acids (Stickland reaction), oxidative deamination from an individual amino acid or reductive deamination of an individual amino acid [50,53]. Lastly, hydrolysis of lipids produces glycerol and long-chain fatty acids. The resulting glycerol can be transformed into pyruvate and then, form acetyl-CoA, acetic acid and other VFAs, while long-chain fatty acids are converted into acetic acid by β-oxidation [49]. Moreover, homoacetogens can produce acetic acid from carbon dioxide and hydrogen through the Wood-Ljungdahl pathway [45,48].

Previous studies have particularly focused on the pathways of methanogens more than that of acetogens and acidogens due to the larger interest in biogas production. Until now, the number of species identified as acidogenic microorganisms is very limited. The most studied phyla able to degrade complex substrates and transform them into VFAs are *Firmicutes*, *Proteobacteria* and *Bacteroidetes* [54]. The variety of species that can perform the acidogenesis step demonstrates that different fermentation products can be obtained depending on the strains present. However, the leading microorganisms will change depending mainly on the substrate, so the substrate would a key element in a bioproduction process of VFAs [44].

During methanogenesis, approximately two thirds of the methane generated comes from acetate. Thus, methanogens are considered competitors of acetogens since they use hydrogen, formate and acetate for growing [55]. The use of acetate as a substrate for growth and for methane formation competes with the accumulation of acids and are carried out by strains that belong to the genera *Methanosarcina* and *Methanosaeta* [54]. Accumulation of acids implies a drop in pH that could lead to a reduction of the methane produced, as methanogens require pH over 6.5 [56]. This reduction, in turn, comes with a greater accumulation of acids, as acetate is not consumed [45]. When using easily biodegradable feedstocks, methanogenesis is the rate-limiting step of the process, as methanogens grow very slowly and need very

specific substrates [57]. The competition between methanogens and acetogens needs further study and it is of great interest for the industrial use of anaerobic fermentation for VFA bioproduction [58]. Strategies promoting the acidogenic step and inhibiting the methanogenesis step must be followed to maximize VFA bioproduction. Most of these strategies can be satisfactorily implemented by tuning the operational conditions of the process, which will be discussed in Section 3.

3. Operational parameters affecting VFA production yield

There are several operational parameters that affect the VFA bioproduction by acidogenic fermentation of waste. But, before starting the analysis, it is important to mention how to quantify the performance of a VFA bioproduction process. In this sense, to quantify the amount of VFAs produced, the literature describes two different parameters: VFA production yield and the degree of acidification. The VFA production yield (expressed in terms of g COD·g^{-1} COD, where COD stands for chemical oxygen demand) can be defined as the amount of VFAs produced divided by the substrate consumed [60]. Other authors, especially when the substrate is a solid waste, define the VFA production yield (expressed in terms of g COD·g^{-1} VSS or g COD·g^{-1} VS) as the amount of VFAs produced divided by the organic matter contained in the solid waste [61, 62] expressed in terms of volatile suspended solids (VSS) or volatile solids (VS). Regarding the degree of acidification, this parameter is calculated by dividing the total VFA concentration in the product effluent and the total initial COD of the substrate [60].

Recently, many researchers have focused their efforts on studying the effect of several operational parameters on VFA production, such as: substrate composition [53,63], pH [64,65], temperature [66,67], volumetric organic loading rate (ORLv) and hydraulic retention time (HRT) [68,69]. The aforementioned parameters affect all the steps of the anaerobic digestion, so they can impact on VFA, hydrogen and methane production and hence it is necessary to understand their influence to maximize VFA accumulation. Most of the mentioned studies have separately analyzed each parameter. Nevertheless, the influence of each one of these parameters on VFA production yield might change depending on the type of carbon source used as substrate regarded in terms of its composition defined as the proportion between carbohydrates, proteins, or lipids [70]. In the current section, we consider the impact of the organic composition of the waste on the VFA production

yield, and then, we sum up the combined influence of some operational parameters: pH, temperature, organic loading rate and HRT according to the organic composition of substrate.

3.1. Organic composition of the waste

There are several types of wastes that can be used as substrates in acidogenic fermentation, including: (i) solid wastes, such as tuna waste [71], mushroom compost [72], apple pomace and winterization oil cake [28], (ii) slurry-like wastes, such as primary sludge from a wastewater treatment plant (WWTP) [73], WAS from a WWTP [28,74], food waste [75], organic fraction of municipal solid waste (OFMSW) [76], maize silage [68], and; (iii) liquid wastes or wastewaters, such as dairy wastewater [24], palm oil mill wastewater [77], sugar industry wastewater [78], olive mill wastewater [26,28,60], glycerol [28,79] and paper mill effluent [64]. The difference between solid and slurry-like waste lies in the solids content. In this work, we consider slurry-like waste to have less than 50% in solids content.

Besides, it makes no sense to compare VFA production yields obtained from different substrates unless the experiments are carried out in exactly the same conditions since several operational parameters might influence VFA yield. Montiel-Jarillo et al. [28] compared different waste streams to determine their acidogenic potential under mesophilic conditions after pretreating the biomass to inhibit methanogenic activity. The larger VFA production yields were obtained when using WAS, olive mill wastewater, winterization oil cake, apple pomace and glycerol (in descending order) [28]. Similarly, Silva et al. [79] previously performed experiments to study the VFA production of several substrates under mesophilic conditions using an inhibitor of methanogenic bacteria. In this case, the substrates that yielded more VFA production (in descending order) were cheese whey, sugarcane molasses, OFMSW, waste glycerol, winery effluent, olive mill wastewater, soapy slurry waste and landfill leachate [79].

As stated in the introduction of Section 3, the organic composition of the waste would affect VFA production yield. Therefore, in this review, the organic matter would be studied in terms of its composition into three different kinds of macromolecules: lipids, carbohydrates and proteins. Very few studies have analyzed VFA production yield from the main organic matter components [53,63]. Yin et al. [63], for example, found that glucose (carbohydrate) led to higher VFA yields in comparison to peptone (protein) and glycerol (lipid hydrolysate) and that a mixture of glucose, peptone and glycerol led to an even larger VFAs yield than glucose alone [63].

In general, lipidic substrates are less preferred than carbohydrates and proteins in fermentation processes. First, hydrolysis of lipids is slower than hydrolysis of carbohydrates and proteins [63]. Secondly, hydrolysis of lipids produces long chain fatty acids (LCFAs) and glycerol. Glycerol can be converted to VFAs, but LCFAs could inhibit the metabolism of anaerobic bacteria, as they adhere to the cell walls, and they tend to decrease nutrient transportation [53]. Finally, the acidogenic microorganisms have more difficulties to produce VFAs from glycerol than from carbohydrates or proteins such as glucose or peptone. Due to their lower degradation rate, lipids tend to accumulate in the degraded waste [63]. The highly reduced nature of carbon atoms in glycerol makes its utilization by microorganisms difficult under fermentative conditions [80]. Nevertheless, the use of acclimatized cultures could be a solution to increase the efficacy of acidogenic fermentation from lipidic waste [81].

Regarding the fermentation of protein-rich substrates, the hydrolysis of proteins could lead to the release of essential nutrients, but the degradation of some amino acids involves hydrogen consumption reactions [53]. Moreover, proteins are less easily biodegraded than carbohydrates, as their structure is more complex than that of carbohydrates. Thus, when using wastes with high protein content, hydrolysis could be the rate-limiting step [82]. Additionally, proteins can contain different aminoacids, and their composition would also

influence VFA production yield. Shen et al. [82] compared the hydrolysis and acidification of tofu (vegetable protein) and white egg (animal protein). They found that white egg conducted to larger VFA yield than that of tofu [82].

A parameter that can be used to study the impact on the substrate composition on VFA production yield is the Carbon to Nitrogen (C/N) ratio. For example, carbohydrates and lipids contribute to increase the C/N ratio of a substrate since they have a higher content in carbon than nitrogen, while proteins tend to decrease the C/N ratio because of their high nitrogen content. An optimal C/N ratio is needed in any anaerobic digestion process to ensure that nutrients are balanced for the maintenance and growth of the bacteria. Moreover, a low C/N ratio could be related to the release of free ammonia or ammonium, which causes acidogenesis inhibition [83]. The optimal range of the C/N ratio for anaerobic digestion is 20–30 [84].

Finally, the combination of different kinds of substrates (co-fermentation) generally leads to higher VFA yields in comparison to the fermentation of different substrates separately. Table 1 presents studies showing that VFA production yield is increased by mixing different substrates. For example, a mixture of carbohydrates and proteins boosts VFA yield, rather than using these two substrates individually: Feng et al. [85] found that adding rice (carbohydrate-rich substrate) to WAS (protein-rich substrate) enhances VFA production in comparison with the fermentation of only WAS [85]. Co-fermentation of more than one kind of substrate can be used to enhance the VFA production yield by balancing the C/N ratio. Besides, co-fermentation processes involve other advantages [86,87]: (i) balancing of micronutrients and moisture, (ii) dilution of inhibitory compounds, (iii) enhancing of the pH-buffer capacity and (iv) providing an active inoculum adapted to the substrate when one or more of the substrates used are waste.

3.2. pH and organic composition of the waste

pH is one of the most important key factors affecting the VFA production yield because it affects the prevalence of acidogenic or methanogenic microorganisms [88,89]. The accumulation of VFA would lead to a decrease in pH, which can inhibit the methanogenic microorganisms [90]. Acidogenic microorganisms can also be inhibited in acidic environments (pH < 5.0) or extremely alkaline conditions (pH>12.0) [91]. Apart from the acidogenesis step, the working pH also has a notable effect on the hydrolysis step [92]. Since the hydrolysis step efficiency is subjected to the complexity of the substrate, optimal pH strongly depends on the nature of the substrate used.

Table 2 shows the pHs reported as optimal to lead to high VFA production yields depending on the substrate used and its organic composition. In general, it can be concluded that acidogenic fermentation of a solid or slurry-like waste, independently of its organic composition, needs an alkaline pH to reach high VFA production yields. Solid or complex wastes usually require an alkaline pH to boost both, hydrolysis and acidogenesis steps [68]. As an example, when primary or WAS from a WWTP is used, the optimal pH range seems to be 8.0–12.0 [88,93]. In this case, alkaline conditions enhance the VFA production since they stimulate the hydrolysis of the sludge by the ionization of the charged groups of the extracellular polymeric substances and the consequent release of fermentable carbohydrates and proteins [91,94]. Moreover, an alkaline environment inhibits the growth of methanogens, such as *Methanobacterium sp.* and *Methanobrevibacter sp.*, avoiding the consumption of the produced VFAs [91,94]. Conflicting results have been reported in studies using food waste as substrate. Dahiya et al. [95] performed several batch tests at pH 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 and found that pH 11.0 led to the higher degree of acidification. In this case, VFA production might be favored by a higher availability of hydrolyzed compounds, similarly to the fermentation of sludge. On the contrary, Zhang et al. [96] carried out batch experiments at pH 5.0, 7.0, 9.0 11.0 and reported that the VFA concentration and production yield were maximized at pH 7. Moreover, Zhang et al. [96] quantified the

Table 1

VFA yield obtained by co-fermentation of substrates with different organic composition.

Primary substrate	Secondary substrate	Main component of the primary substrate	Main component of the secondary substrate	VFA yield of the primary substrate	VFA yield of the mixture (primary and secondary substrates)	Increment of the VFA yield obtained by co-fermentation in comparison to primary substrate fermentation*	Reference
Primary sludge from a WWTP	Oleic acid	Proteins	Lipids	119 mg COD g^{-1} VS **	176 mg COD g^{-1} VS* *	48%	[86]
Secondary sludge from a WWTP	Oleic acid	Proteins	Lipids	41 mg COD g^{-1} VS **	160 mg COD g^{-1} VS* *	288%	[86]
Secondary sludge from a WWTP	Aged refuse	Proteins	Carbohydrates	83 mg COD g^{-1} VSS	184 mg COD g^{-1} VSS	122%	[61]
Secondary sludge from a WWTP	Rice	Proteins	Carbohydrates	101 mg COD g^{-1} VSS	520 mg COD g^{-1} VSS	413%	[85]
Pretreated secondary sludge from a WWTP	Potato peel waste	Proteins	Carbohydrates	132 mg COD g^{-1} VS	344 mg COD g^{-1} VS	160%	[101]
Primary sewage sludge from a WWTP	Organic waste	Proteins	Carbohydrates	250 mg COD g^{-1} VS	301 mg COD g^{-1} VS	20%	[62]
Pretreated secondary sludge from a WWTP	Food waste	Proteins	Carbohydrates and proteins	132 mg COD g^{-1} VS	282 mg COD g^{-1} VS	113%	[101]

*The increment was calculated by dividing the difference between the VFA yield of the mixture and the VFA yield of the primary substrate, by the VFA yield of the primary substrate, expressed as %.

* *VFA yields calculated by the authors from the reported data

Table 2

Optimal pH reported to maximize VFA production yields for different substrates.

Substrate	Main component of the substrate	Physical state	pH range studied	Optimal pH	Reference
Secondary sludge from a WWTP	Proteins	Slurry-like	7.0–12.0	11.0 for hydrolysis 9.0 for acidification	[93]
Primary sludge from a WWTP	Proteins	Slurry-like	3.0–11.0	10.0	[102]
Slaughterhouse wastewater	Proteins	Liquid	5.5 and 10.0	10.0	[64]
Gelatin-rich wastewater	Proteins	Liquid	4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0	6.0	[98]
			5.0 and 7.0	7.0	[99,100]
Mushroom compost	Carbohydrates	Solid	4.0–12.0	10.0	[72]
OFMSW	Carbohydrates	Slurry-like	5.5 and 10.0	10.0	[64]
Maize silage	Carbohydrates	Slurry-like	5.0 and 11.0	11.0	[68]
Food waste	Carbohydrates	Slurry-like	5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0	10.0	[95]
			5.0, 7.0, 9.0 and 11.0	7.0	[96]
			4.0, 5.0, 6.0	6.0	[75]
Cheese whey	Carbohydrates	Liquid	3.5–6.0	5.3–5.5	[65]
			5.0 and 11.0	5.0	[68]
Paper mill wastewater	Carbohydrates	Liquid	5.0–6.0	5.5–6.0	[65]
			5.5 and 10.0	10.0	[64]
Winery wastewater	Carbohydrates	Liquid	5.5 and 10.0	10.0	[64]
Citrus wastewater	Carbohydrates	Liquid	4.0, 5.0, 6.0 and 7.0	7.0	[70]
Crude glycerol	Lipid derived product	Liquid	5.5 and 10.0	10.0 at mesophilic conditions 5.5 at thermophilic conditions	[64]
Tuna waste	Proteins and carbohydrates	Solid	5.0–10.0	8.0	[71]
Microalgae biomass	Proteins, lipids and carbohydrates	Slurry-like	5.0 and 11–0	11.0	[68]
Olive mill solid waste	Carbohydrates and lipids	Slurry-like	5.0 and 9.0	9.0	[103]

degree of solubilization of the substrate and found that pH 7.0 conducted to the maximum degree of solubilization for carbohydrates, proteins and lipids in comparison to the rest of pH conditions tested. These differences might arise from the different organic composition of the food waste, which was not reported by Dahiya et al. [95].

Regarding liquid wastes, the optimal pH varies depending on its organic composition. Hydrolysis and acidification of lipids and proteins need a different pH from simple carbohydrates to achieve high VFA production yield [97]. Fermentation of monomer-rich substrates, which do not require such an extensive hydrolysis, seems to be more favorable at low pHs. Methanogens are active in a pH range between 6.5 and 8.2 and its activity is inhibited at higher or lower pHs [56]. However, acidogens can grow in a wider range of pH than metanogens. In this way, Table 2 shows that carbohydrate-rich liquid wastes, such as cheese whey [65,68] and citrus wastewater [70] lead to higher VFA production yield

at neutral pH in comparison to acidic or basic conditions. When using paper mill wastewater, Bengtsson et al. [65] reported that neutral pH was more convenient, while Garcia-Aguirre et al. [64] found that alkaline conditions result in higher VFA production. Despite being the same type of waste, they might have a very different composition. Garcia-Aguirre et al. [64] also found that alkaline pH leads to higher VFA production yields than lower pH for winery wastewater.

From the aforementioned studies, it can be concluded that for liquid wastewaters that are mainly composed of carbohydrates, the optimal pH for VFA production depends strongly on the complexity of the carbohydrates forming the waste. As a general rule, alkaline pH would be needed when using wastewaters with complex carbohydrates and neutral pHs are more favorable for liquid wastes with simpler carbohydrates that do not need intensive hydrolysis.

There are fewer studies on optimal pH conditions for protein-rich or

lipid-rich liquid substrates. Garcia-Aguirre et al. [64] found that the optimal pH when using crude glycerol as a substrate was 10.0 in mesophilic conditions and 5.5 in thermophilic conditions. In the case of protein-rich substrates, one might find different results depending on the type of waste. Slaughterhouse wastewater was reported to yield more VFAs at alkaline conditions [64], while gelatin-rich wastewater produced better results at neutral pH [98–100]. Probably, these differences are due to the different complexity of the proteins forming the waste. It seems that the higher the complexity of the proteins, the higher the optimal pH.

Recently, novel pH-stepwise control processes are being considered as a promising alternative to enhance VFA production from a substrate mainly composed by complex proteins [73,93]. In pH-stepwise processes, pH is maintained firstly at alkaline levels to promote the hydrolysis step, and then, is lowered to a pH level closer to neutrality to perform the acidogenesis. Wang et al. [73] demonstrated that a pH stepwise control strategy that consisted in maintaining pH at 11.0 during the early stage and then keeping pH at 9.0 during the rest of the fermentation led to the higher VFA yields from primary sludge. pH 11.0 at the beginning increased the available soluble proteins and carbohydrates while inhibiting methanogenic activity and, subsequently, pH 9.0 increased the abundance and diversity of acidogenic bacteria while keeping high levels of soluble substrates.

During acidogenic fermentation, the accumulation of VFAs could lead to a severe drop of pH. At low pH levels, acids exist as undissociated molecules, which could pierce the cell membrane and reduce the microbial activity by directing the energy generated to cell maintenance [104]. Therefore, pH control and extraction of the produced acids would serve to maintain a stable operation of the process. On the contrary, pH control would imply higher process costs due to the addition of acids and bases to adjust pH and the automatic control loop installation. In the design and scaling up of a VFA production process, it would be necessary to study if the increment of VFAs produced after implementing a pH control loop compensates the costs derived from pH control.

3.3. Temperature and organic composition of the waste

Temperature is another relevant operational parameter since it influences the metabolic rates, enzymatic activities and microorganisms' growth and decay rates [105]. For this reason, the optimal temperature for enhancing VFA yield depends on the microbial consortium composition and the organic composition of the waste. Several studies have carried out experiments on VFA production using different substrates at different temperature ranges, namely psychrophilic (4–20 ºC), mesophilic (20–50 ºC), thermophilic (50–60 ºC) and hyperthermophilic (60–80 ºC) conditions [64,66,67,105–108].

Most of the authors agree on the mesophilic range being more favorable in terms of VFA production yield in comparison to psychrophilic conditions [67,105,109,110]. Fernández-Domínguez et al. [105], for example, found that mesophilic conditions when using OFMSW as substrate led to higher VFA yields compared to psychrophilic temperatures. Similarly, if the substrate is WAS, increasing the temperature from psychrophilic to mesophilic ranges boosts the hydrolysis rate, making carbohydrates and proteins more available and rising the activity of the acid-forming enzymes, enlarging, therefore, VFA production yield [67, 110]. Hydrolytic enzymes also show higher activities and the abundance of acidogenic bacteria was richer at mesophilic conditions compared to psychrophilic conditions [110]. Due to all of this, there is a clear agreement on the fact that mesophilic conditions are more favorable to VFA production rather than psychrophilic conditions.

When comparing the effect of mesophilic temperatures with thermophilic and hyperthermophilic temperatures on VFA production yield from a solid or a slurry-like waste, the more convenient conditions are dependent on the organic composition of the waste. On one hand, some studies found that mesophilic conditions were more favorable than thermophilic conditions for the acidogenic fermentation of different

carbohydrate-rich substrates such as: food waste [106,108], OFMSW [64,105] and cow manure mixed with maize silage [111]. Nevertheless, several studies found higher VFA yields at thermophilic conditions than at mesophilic conditions when using protein-rich substrates such as: WAS [64,66,112,113] and meat and bone meal [64]. Based on the above analysis, it can be concluded that mesophilic temperatures boost VFA yields with carbohydrate-rich solid substrates rather than thermophilic conditions; and thermophilic temperatures enhance VFA production with protein-rich substrates in comparison with mesophilic conditions. The reason for the difference is that increasing the temperature from mesophilic to thermophilic range boosts the hydrolysis of the substrates by rising the key hydrolases activities [66], despite the key acid-forming enzymes have been reported to have higher activities at mesophilic conditions rather than thermophilic conditions [67]. Proteins are characterized by their low biodegradability because of their complex structure (in comparison to carbohydrates) so the hydrolysis is considered the rate-limiting step in VFA synthesis from proteins [82]. Thus, carbohydrate-rich solid substrates that do not need such extensive hydrolysis as proteinic substrates would have higher VFA yields at mesophilic conditions than at thermophilic conditions.

Less research is reported regarding the effect of temperature on the acidogenic fermentation of liquid substrates. Most of the studies of VFA synthesis are carried out in the mesophilic range [24,65,68,97,114]. Nevertheless, thermophilic conditions have been reported to be more optimal in terms of VFA production from several substrates such as gelatin-rich wastewater (protein-rich substrate) [98], slaughterhouse wastewater (protein-rich substrate) [64], paper mill wastewater (carbohydrate-rich substrate) [64] and winery wastewater (carbohydrate-rich substrate) [64]. Conversely, mesophilic temperatures are more convenient when employing glycerol as substrate (lipid-rich substrate) [64]. Further research about the influence of temperature on VFA production from liquid wastes is needed.

Besides the substrate, the optimal temperature could also depend on the microbial population. Some bacterial consortia are more sensitive to temperature changes than others are [115]. Thus, inconsistent results between studies that use substrates with similar organic composition could be caused by the inoculum used in each case [116], specifically when the acidogenesis is the rate-limiting step. In addition, synergic effects of the temperature and other process parameters on the VFA production yield have been described, namely pH [64] and substrate composition [76].

3.4. Volumetric organic loading rate, hydraulic retention time and organic composition of the waste

The volumetric organic loading rate (ORLv), usually expressed as g COD·L^{-1}·d^{-1}, is a parameter that affects VFA production yield since it represents the amount of substrate available per liter of reactor and per day to be transformed into VFAs. However, to consider the effect of the amount of inoculum added over the VFA production yield, a specific organic loading rate (OLRs) (expressed as g COD·g^{-1} VSS$_{biomass}$·d^{-1}) should be used.

For a fixed working volume of reactor and a fixed substrate concentration in the influent, the OLRv is inversely proportional to the HRT. OLRv can be increased in two ways: by increasing the influent substrate concentration or by lowering the HRT. Since this review is dealing with waste streams as substrate in acidogenic fermentation, its concentration cannot easily be changed, so OLRv is normally regulated by tuning the HRT. Therefore, the impact of both OLRv and HRT is evaluated together in this section, focusing on the effect of OLRv on the VFA production yield.

In general terms, increasing the OLRv rises VFA production, as there is more substrate available for the microorganisms. Furthermore, methanogen growth is slower than acidogen growth, so raising the OLRv is also a way to wash out the methanogens from the reactor [117,118]. However, at very high OLRv, the reactor operation could become

unstable due to a sharp drop of pH caused by a high VFA production that can cease the activity of acidogenic microorganisms [119–121].

The optimal OLRv is more dependent on the complexity of each one of the components of the waste rather than the proportion of the different fractions (carbohydrates, proteins, lipids) on it. When working with complex substrates (such as WAS) or complex carbohydrates (like food waste), hydrolysis stage would be the rate-limiting step, so a larger HRT of fermentation is needed. Thus, OLRv must be keep low when working with rather complex proteins or carbohydrates to avoid the increase in the broth viscosity produced by the accumulation of non-hydrolyzed components, which would lead to a low VFA production due to mass transfer limitations [69,122]. Jankowska et al. [68] compared the VFA production yield obtained at different HRT and pH from four different substrates, complex/simple carbohydrate-rich substrates (maize silage/glucose), complex/simple protein-rich substrate (microalgae biomass/whey). They concluded that VFA production yield strongly depends on the complexity of each one of the components of the waste and that an alkaline pH enhances VFA yield more than an increase in the HRT [68]. Alternately, several pretreatments of wastes described in the literature allow to apply larger OLRv by enhancing the hydrolysis step efficiency [123].

There is no doubt that the application of high OLRv would help to wash out the methanogens, but if this ORLv is high enough, some acidogenic microorganisms would be also washed out the reactor. Therefore, the OLRv affects, at the end. all the microbial communities present in the reactor [115,117] and, in consequence, the VFA composition distribution, which is discussed in Section 4.

4. Operational parameters affecting VFA distribution

In Section 3, we analyzed the influence of different operational parameters together with the organic composition of the waste on the VFA production yield. However, the impact of these parameters on the production of a particular VFA or a mixture of VFAs is even more difficult to determine. As we mentioned before, acidogenic fermentation is a complex process where numerous biological reactions take place. Moreover, when using MMC, the different microorganisms present in the reactor could follow different metabolic pathways and could also vary with applied operational conditions. Thus, the spectrum of obtained products from acidogenic fermentation by MMC could be very wide. Therefore, more knowledge is needed to understand how to drive the acidogenic fermentation process to obtain a specific VFA or a mixture of VFAs.

4.1. Organic composition of the waste

Some authors studied the influence of the organic composition of the waste on the VFA composition of the effluent form acidogenic fermentation without considering any other operational conditions. For example, Alibardi and Cossu [53] performed batch fermentation experiments using mixtures of four fractions of different organic wastes and analyzed the produced VFAs. They only found a correlation between butyric acid production and the chemical composition of the wastes (in terms of percentage of carbohydrates, lipids, and proteins). Carbohydrate content seemed to be the main factor influencing butyric acid concentration. Nevertheless, the correlation was weak, since R^2 value was 0.809 [53]. Ma et al. [101] obtained similar results by carrying out fermentation batch experiments using mixtures of WAS and potato peel or food waste in different ratios. In all the trials, acetic acid was the main product and when the carbohydrate content in the waste was higher than that of proteins, butyric acid and ethanol concentrations increased while propionic and valeric acid decreased. They also correlated propionic, butyric and valeric acid concentrations with lipid, starch, and protein consumption but, again, R^2 values were quite low (between 0.62 and 0.81) [101]. Yin et al. [63] performed batch tests using glucose, peptone and glycerol as model compounds of simple carbohydrates, proteins and lipids respectively, to elucidate more clearly which

component of organic matter is responsible for the formation of each VFA. It was observed that butyric acid was the predominant compound produced from glucose, acetic acid was the main product from peptone and propionic acid the main one when using glycerol. Additionally, when a mixture of the three components was used, the VFA composition of the effluent was not the result of an additive effect of each component since propionic acid proportion would be expected to be the 32% of the total VFAs (in a COD basis) and it was actually the 40% of the total VFAs [63]. Once again, these studies suggest that the organic composition of the waste is the main factor that influences the VFA production yield and the composition of the obtained VFAs, but other parameters may also affect. Therefore, in the following sections, the interactional effect of pH, temperature and bacterial composition with the organic composition of waste on the VFA composition is revised. Furthermore, more studies using model compounds of carbohydrates, proteins, and lipids as substrates, but working in continuous or semicontinuous conditions are needed to evaluate the effect of organic composition of the waste on VFA composition in long term processes.

4.2. pH and organic composition of the waste

The effect of pH on VFA composition is by far the most studied among the different operational parameters. In this section, the joint impact of pH and organic composition of the waste on the composition of the VFA rich stream produced is analyzed. Table 3 collect VFA distributions obtained in the fermentation of wastes with different organic composition.

According to the reviewed studies, there is agreement on the VFAs obtained from protein-rich substrates at different pH. pH lower than 5.0 leads to propionic acid while a pH between 5.0 and 11.0 leads to acetic acid [98,124,125]. Liu et al. [124] reported that a pH of 12.0 produced butyric acid as a main component. The rest of the VFA components with lower presence vary among the different studies [98,124]. In any case, it can be affirmed that pH is an appropriate tool to change VFA composition when using protein-rich substrates.

Nevertheless, carbohydrate-rich substrates yielded very different VFA compositions in the different studies shown in Table 3. On the one hand, Atasoy et al. [114] performed experiments using glucose as substrate (carbohydrate) and determined the VFA composition obtained using different types of inoculums. The main VFA component was butyric acid independently of the inoculum used for a wide range of pH conditions (pH between 5.0 and 10.0) [114]. On the other hand, pH has a strong effect when using substrates mainly composed of complex carbohydrates, namely paper mill wastewater and cheese whey [65]. In paper mill wastewater fermentation experiments, an increase of pH from 4.9 to 6.0 changed the main VFA components from acetic acid (49%), butyric acid (18%) and propionic acid (13%); to butyric acid (33%), propionic acid (23%) and acetic acid (22%). An increment of pH from 5.3 to 6.0 in cheese whey acidogenic fermentation experiments, shifted the main VFA components from acetic acid (51%), butyric acid (24%) and propionic acid (19%); to propionic acid (41%), acetic acid (31%) and butyric acid (10%) [65].

Table 3 collects as well studies of VFA compositions obtained from fermentation of wastes that are formed of carbohydrates and proteins [51,108,126,127] and carbohydrates and lipids mixtures [97,103]. In these cases, the substrate heterogeneity makes it even more complicated to link the organic composition and the pH with the final VFA composition obtained. Luo et al. [126] performed experiments of different pH of a mixture of WAS (protein-rich substrate) and wine vinasse (carbohydrate-rich substrate) (1:1, in COD proportions). The addition of wine vinasse changed the main VFA component at different pH ranges: acetic acid (from pH 3.0–5.0), acetic and propionic acids (from pH 6.0–9.0) and acetic acid (pH 10.0) [126]. Food waste (carbohydrate-rich and protein-rich substrate) is one of the most studied substrates. Different studies arrived at different VFA distributions at similar pH conditions [51,108,127]. Feng et al. [51] discussed the different pathways that can

Table 3

Effect of pH on VFA distribution by using wastes with different organic composition.

Substrate	Main component of the substrate	Inoculum used	pH	Main products (in descending order) (% of the total VFA)	Reference
Gelatin-rich wastewater	Proteins	Methanogenic anaerobic sludge	4.0	Propionic acid (32%) and acetic acid (15%)	[98]
			4.5	Propionic acid (27%) and acetic acid (18%)	
			5.0	Acetic acid (23%) and propionic acid (20%)	
			5.5	Acetic acid (25%), isobutyric acid (13%), butyric acid (13%), isovaleric acid (13%), propionic acid (12%) and valeric acid (12%)	
			6.0	Acetic acid (28%), butyric acid (16%), isobutyric acid (13%) and propionic acid (13%)	
			6.5	Acetic acid (32%) and butyric acid (21%)	
			7.0	Acetic acid (35%) and butyric acid (22%)	
Sewage sludge from a brewery	Proteins	Pretreated sewage sludge (thermal pretreatment to inactivate methanogens)	3.0	Propionic acid (56%) and acetic acid (44%)*	[124]
			5.0	Acetic acid (31%), butyric acid (24%) and valeric acid (20%)*	
			7.0	Acetic acid (26%), propionic acid (21%), butyric acid (16%) and valeric acid (15%)*	
			9.0	Acetic acid (44%) and propionic acid (19%)*	
			11.0	Acetic acid (44%) and propionic acid (19%)*	
			12.0	Butyric acid (37%) and acetic acid (31%)*	
Secondary sludge from a WWTP	Proteins	Slurry anaerobic biomass	7.0	Acetic acid (47%)	[125]
			10.0	Acetic acid (56%)	
Heat-alkaline pretreated secondary sludge	Proteins	Slurry anaerobic biomass	7.0	Acetic acid (57%)	[125]
			10.0	Acetic acid (65%)	
Glucose solution	Carbohydrates	Small granular anaerobic biomass	5.0	Butyric acid (58%) and acetic acid (24%)*	[114]
			8.0	Butyric acid (47%) and acetic acid (35%)*	
			10.0	Butyric acid (56%) and acetic acid (42%)*	
		Large granular anaerobic biomass	5.0	Butyric acid (52%) and acetic acid (39%)*	
			8.0	Butyric acid (50%) and acetic acid (33%)*	
			10.0	Butyric acid (58%) and acetic acid (39%)*	
		Slurry anaerobic biomass	5.0	Butyric acid (46%) and acetic acid (14%)*	
			8.0	Butyric acid (55%) and acetic acid (15%)*	
			10.0	Butyric acid (47%) and acetic acid (44%)*	
Paper mill wastewater	Carbohydrates	Acidogenic biomass from a reactor treating paper mill wastewater	4.9	Acetic acid (49%), butyric acid (18%) and propionic acid (13%)	[65]
			6.0	Butyric acid (33%), propionic acid (23%) and acetic acid (22%)	
Cheese whey	Carbohydrates	Acidogenic biomass from a reactor treating paper mill wastewater	5.3	Acetic acid (51%), butyric acid (24%) and propionic acid (19%)	[65]
			6.0	Propionic acid (41%), acetic acid (31%) and butyric acid (10%)	
Secondary sludge from a WWTP + wine vinasse	Proteins and carbohydrates	No inoculum added (endogenous microorganisms from the substrate)	3.0	Acetic acid (52%) and butyric acid (36%) *	[126]
			4.0	Acetic acid (41%), butyric acid (24%) and propionic acid (21%) *	
			5.0	Acetic acid (37%), propionic acid (31%) and butyric acid (19%) *	
			6.0	Propionic acid (40%) and acetic acid (38%) *	
			7.0	Propionic acid (45%) and acetic acid (38%) *	
			8.0	Propionic acid (42%) and acetic acid (40%) *	
			9.0	Acetic acid (43%) and propionic acid (40%) *	
			10.0	Acetic acid (38%) and propionic acid (33%) *	
Food waste	Carbohydrates and proteins	Mesophilic anaerobic digested sludge	5.0	Acetic acid (60%) and butyric acid (31%)	[108]
			6.0	Butyric acid (53%) and acetic acid (24%)	
			7.0	Butyric acid (43%) and acetic acid (34%)	
	Carbohydrates and proteins	Sludge from anaerobic digester	3.2	Lactic acid (87%)	[51]
			4.0	Lactic acid (81%)	
			4.2	Lactic acid (81%)	
			4.5	Lactic acid (57%) and acetic acid (25%)	
			4.7	Butyric acid (56%) and acetic acid (19%)	
			5.0	Butyric acid (40%) and acetic acid (40%)	
			6.0	Butyric acid (40%) and valeric acid (29%)	
Food waste + mature compost	Carbohydrates and proteins	Biomass from anaerobic digester treating food waste	6.0	Hexanoic acid (43–47%), butyric acid (21–25%) and acetic acid (22–23%)	[127]
			7.0	Acetic acid (36–38%), hexanoic acid (24–26%) and butyric acid (18–20%)	
Olive oil mil waste	Carbohydrates and lipids	Anaerobic biomass acclimatized to secondary sludge	5.0	Acetic acid (60%) and propionic acid (23%)	[103]
			9.0	Acetic acid (79%)	
Dairy wastewater	Lipids and carbohydrates	Anaerobic sludge treating synthetic dairy wastewater	4.0	Propionic acid (38%) and acetic acid (18%)	[97]
			4.5	Propionic acid (34%) and acetic acid (20%)	
			5.0	Propionic acid (28%) and acetic acid (26%)	
			5.5	Acetic acid (28%), propionic acid (18%) and butyric acid (13%)	
			6.0	Acetic acid (33%), butyric acid (14%) and propionic acid (13%)	
			6.5	Acetic acid (34%), butyric acid (14%) and propionic acid (12%)	

* The VFA compositions were obtained (and calculated in some cases) from graphically presented results.

take place at different pH conditions and would explain the VFA compositions obtained. Homolactic fermentation (following EMP and PPP pathways) takes place at pH 3.2–4.5 leading to lactate production. Heterolactic fermentation (following PK pathway) occurs at pH 3.2–5.0 leading to lactate and ethanol production. Ethanol type fermentation (following EMP pathway) takes place at pH 4.5 and directs fermentation to ethanol, acetate and H_2. Ethanol fermentation (following ED pathway) takes place at pH 4.4–6.0 and yields ethanol. Heterolactic fermentation (following Bifidus pathway) occurs at pH 4.5–5.0 leading to acetate and lactate. Acetone-butanol-ethanol fermentation (following EMP pathway) takes place at pH 4.7–4.9 leading to acetone, butanol, ethanol and H_2. Butyrate fermentation (following EMP and PPP pathways) occurs at pH 5.0 and leads to butyrate, acetate and H_2. Finally, mixed acid fermentation (following EMP and PPP pathways) takes place at pH 6.0 and yields acetate, propionate, butyrate, valerate and H_2 [51]. Since several pathways can take place at the same pH conditions, the differences between organic composition of the food waste used in the reported studies might explain the different VFA distributions obtained [51,108,127].

Moreover, the pH effect on the VFA composition also depends on the microbial population present and, at the same time, pH influences the microbial community structure. Thus, the pH could yield different VFA composition depending on the culture history. As an example, Mohd-Zaki et al. [128] carried out glucose fermentation experiments using two different pH regulation modes (progressive mode and reset mode) which led to non-identical VFA compositions at the same pH levels. Progressive pH regulation led to a gradual change from butyric and acetic acids to acetic acid and ethanol as main components as pH increased, while resetting pH regulation caused a clearly defined change from acetic and butyric acids to acetic acid and ethanol as main components when pH exceeded 6.5 [128].

4.3. Temperature and organic composition of the waste

Less studies have been published about the effect of the temperature on the VFA distribution. Each VFA can be produced by different strains, and each of these species can have a different optimal growth temperature. Thus, the proportions of each VFA produced can vary with temperature.

From the results collected in Table 4, it can be gathered that VFA distribution for most of the substrates is not as dependent on temperature as it is on pH. The reported results show that the main VFA component does not vary with the temperature used, from psychrophilic to thermophilic ranges, for both protein-rich substrates [66,67,110] and carbohydrate-rich substrates [105,129]. The composition of the second major and subsequent VFAs exhibited more variations with temperature in some studies [110,129]. Nonetheless, several studies that used food waste as substrate, reported different VFA compositions depending on the temperature. Lim et al. [69] carried out food waste fermentation in a semicontinuous reactor at different temperatures and found that the main VFAs produced were propionic, acetic and valeric acids at 25 ℃; acetic and propionic acids at 35 ℃ and acetic and hexanoic acids at 45 ℃. Later, Jiang et al. [108] performed batch experiments of food waste fermentation at different temperatures and pointed out that the main VFAs produced were acetic and propionic acids at 35 ℃; propionic and acetic acids at 45 ℃ and butyric acid at 55 ℃.

4.4. Bacterial composition in the anaerobic reactor and organic composition of the waste

Regarding bacterial composition, numerous studies analyzed the bacteria present in the anaerobic reactors to link them to the production of different VFAs. Despite the fact that some species of bacteria are known to be responsible for the production of specific types of VFAs

Table 4
Effect of temperature on VFA distribution by using wastes with different organic composition.

Substrate	Main component of the substrate	Inoculum used	Temperature (ºC)	Main products (in descending order) (% of the total VFA)	Reference
Ultrasonic-pretreated secondary sludge from a WWTP	Proteins	No inoculum added (endogenous microorganisms from the substrate)	10, 20, 37, 55	Acetic acid (47–49%), isovaleric acid (16–23%) and propionic acid (9–15%)	[67]
Secondary sludge from a WWTP	Proteins	No inoculum added (endogenous microorganisms from the substrate)	15	Acetic acid (41%) and propionic acid (22%)	[110]
			30	Acetic acid (49%), isovaleric acid (14%) and propionic acid (13%)	
Dewatered sludge	Proteins	No inoculum added (endogenous microorganisms from the substrate)	35	Acetic acid (45%) and isovaleric acid (20%)	[66]
			55	Acetic acid (51%) and isovaleric acid (22%)	
Palm mill oil effluent	Carbohydrates	Sludge from the treatment of palm oil mill effluent	30	Acetic acid (37%) and propionic acid (26%) *	[129]
			40	Acetic acid (43%) and propionic acid (25%) *	
			55	Acetic acid (58%) and propionic acid (14%) *	
OFMSW	Carbohydrates	No inoculum added (endogenous microorganisms from the substrate)	20, 35, 45, 55, 70	Acetic acid (28–33%), butyric acid (25–29%) and propionic acid (22–26%)	[105]
Food waste	Carbohydrates and proteins	Anaerobic sludge	25	Propionic acid (44–46%), acetic acid (20–22%) and valeric acid (18–19%)	[69]
			35	Acetic acid (30–33%) and propionic acid (25–28%)	
			45	Acetic acid (49–50%) and hexanoic acid (23–25%)	
	Carbohydrates and proteins	Mesophilic anaerobic sludge	35	Acetic acid and (37%) and propionic acid (31%)	[108]
			45	Propionic acid (38%) and acetic acid (33%)	
			55	Butyric acid (81%)	

* The VFA compositions were obtained from graphically presented results.

[17], the number of known acidogenic microorganisms is still limited [77].

Nevertheless, the bacterial communities present in a MMC strongly depend on the organic composition of the substrate [130–132]. Moreover, the bacterial composition could also change with pH [51,89,128, 133] as well as other operational parameters such as temperature [134]. Some authors analyzed the combined effect of the pH and the bacterial community composition and structure on the VFA compositions obtained [22,114]. Atasoy et al. [114] evaluated the VFA composition obtained from glucose fermentation at different pHs when using three different inoculum types. They found that inoculum structure did not affect VFA composition while pH was determinant, as discussed before. At the same time, the dominant microbial community affected VFA production more than VFA composition [114]. Wang et al. [22] performed experiments of food waste fermentation at different pH conditions with two types of inoculums. pH did not affect VFA composition in the reactors working with anaerobic activated sludge, since acetic and butyric acids where the main components in all pH conditions. However, when the inoculum was aerobic activated sludge, pH 4 and uncontrolled pH yielded acetic and propionic acids, while pH 5 and 6 led to butyric and acetic acids [22].

Numerous studies carried out acidogenic fermentation experiments of different substrates and analyzed the VFA compositions obtained as well as the bacterial communities at phylum, class, order, family, and genus level [68,77,89,91,114,132]. Nevertheless, the link of each VFA produced with the microorganisms responsible for its synthesis is still not clear. Further work is needed to understand the relationship of microbial communities and VFA composition.

4.5. Prediction of VFA distribution by energy-based metabolic models

As explained above, the VFA composition of the effluents of an acidogenic fermentation process could depend on multiple factors, making it very difficult to predict what composition can be obtained depending on the experimental conditions applied. Against this background, mathematical modelling of the fermentation processes could be a powerful tool to predict VFA production. The aim of this section in not to support the interpretations gathered in the previous sections for each operational parameter and its correlation with organic composition. Here, the energy-based metabolic models are presented as the best mathematical tool available up to date to predict the VFA composition. To the best of our knowledge, the energy-based metabolic models developed so far only consider simple molecules as substrate and pH as the variables that affect VFA composition.

In thise sense, several types of models have been proposed to achieve this objective. The first proposed models were based on the stoichiometry of the fermentation reactions, considering it constant and not considering the possible variations due to changes in operational conditions [135]. From that point, several corrections have been applied to consider operational conditions by using variable stoichiometry models [136,137]. However, these models do not have enough predictive capacity. They are very similar to ASM-family models that were created to describe aerobic processes, which are usually controlled by kinetics [138]. Nevertheless, anaerobic processes supply low energy, and their conversion is limited by the thermodynamic equilibrium [139]. Therefore, energy-metabolic modelling seems to be, nowadays, the best way to predict VFA composition in fermentation processes.

Energy-based metabolic models assume that in anaerobic fermentation processes, microorganisms would follow those pathways that return more net energy from the substrate and consequently lead to biomass growth. Thus, the VFAs linked to a higher ATP yield would be the dominant products of the fermentation [32]. Some studies developed models to predict fermentation product spectrum of glucose [140], proteins [141] and co-fermentation of carbohydrates and proteins [32] by MMC.

González-Cabaleiro et al. [140] elaborated a model considering that one single hypothetical microbial population was capable of carry out all the most important metabolic pathways from glucose. It was the first model capable of predicting the effect of pH on the product spectrum by considering the role of the different electron carriers (ferredoxin, NAD (H) and FAD(H_2)) and a model for the transport of solutes across the cell membrane. The model predicted high yields of butyric acid at low pH, high yields of acetic acid and ethanol at high pH and acetic acid and propionic acid as secondary product at neutral pH [140]. The main difference with the experimental study they took as reference is the presence of acetic and butyric acids as main products at low pH, not only butyric acid [140,142]. Later, Regueira et al. [143] improved this model by including electron bifurcation in the butyrate synthesis pathway and homoacetogenesis that consumed part of the H_2 produced. After this modification, they succeed predicting both acetic and butyric acids at low pH [143].

Subsequently, Regueira et al. [141] built an energy-based metabolic model for predicting VFA formation from proteinic substrates. In that study [141], they considered the same approach that González-Cabaleiro et al. [140] held previously. In that case, it is again assumed that a virtual microorganism can perform all the pathways and that protein consumption can be limited in case it is not thermodynamically favorable. Moreover, the different amino acids could interact among them limiting the degradation of others, mainly provoked by NADH competition. Net NADH balance must be neutral since in absence of oxygen, there is no electron acceptor. This balance can be affected by external pH, so this model is able to predict the preferred pathways that lead to different VFAs for the fermentation of a substrate with a defined amino acid composition at different pH [141].

Since wastes are made up of more than one component, Regueira et al. [32] proposed a model for fermentation of a mix with different ratios of carbohydrates and proteins by using glucose and gelatin as model substrates. The addition of glucose to casein fermentation adds a source of reductive power, changing the NADH balance and the pathways that maximize ATP yield. In that case, protein consumption rate is assumed to be lower than glucose consumption based on experimental results. One of the main limitations of this model is the little information available about the amino acid profile of the gelatin. If it is used as a design tool, the amino acid composition of the proteins used must be previously determined [32].

In view of all the previously mentioned research, energy-based metabolic models seem to be the best method to mechanistically explain fermentation processes. Experimental data can be used to prove the predictable capacity of these models, but do not allow us to extrapolate to operational conditions different to those investigated. Nevertheless, mechanistic models allow us to explore VFA distribution under different operational conditions by changing those defined as environmental conditions. Future work is needed to build models for more complex substrates and introduce the hydrolysis step in the metabolic networks.

5. Example of application of bioproduced VFAs: how the composition of the VFAs determines the type of polyhydroxyalkanoate (PHA) produced

Polyhydroxyalkanoates (PHAs) are some of the multiple products that can be obtained from VFAs. PHAs can be defined as biodegradable polyesters, composed of hydroxyalkanoic acids, and synthesized by bacteria [144,145]. There are more than 150 different hydroxyalkanoic acids that can be integrated into the PHAs' molecular structure. Consequently, the properties of the resulting polymers depend on their composition [144]. PHAs are usually classified into two different groups depending on the number of carbon atoms of their monomers. Short chain length PHAs (scl-PHAs) are composed of monomers with 3–5 carbon atoms while medium chain length PHAs (mcl-PHAs) contain monomers with 6–14 carbon atoms [146]. scl-PHAs are characterized for having a high degree of crystallinity and high melting and low glass

transition temperatures, making them fragile and difficult-to-deform materials. Conversely, mcl-PHAs are less crystalline so they are elastomeric materials that can be used in high value-added applications [146]. Their glass transition temperature is below zero and their melting point is lower than scl-PHAs, so it is easier to mold them [147]. Thus, PHAs with a wide range of properties can be produced and a great number of potential applications can be found for these polymers, as pointed out by Prajapati et al. [148]. Nevertheless, PHAs have gained attention mainly because they are biocompatible and fully biodegradable, and their mechanical properties are comparable to petroleum-based plastics such as polyethylene and polypropylene, as reported by Anjum et al. [149].

Today, PHA production at an industrial scale is carried out by using pure cultures or genetically modified strains. The main disadvantage of PHAs production processes with pure cultures is that they usually require highly pure substrates [146]. Therefore, the PHAs obtained are not commercially competitive against fossil-fuel-derived plastics since their production costs are between 2.0 and 4.6 times higher than the conventional plastics costs [150]. Besides, pure cultures need large quantities of co-substrates to obtain polymers containing a relatively low fraction of monomers different from 3-hydroxybutyrate (3HB) [151] possible to produce PHAs by chemical synthesis or by employing genetically modified plants, but these processes have been considered less interesting for the industry.

On the one hand, PHAs can be chemically synthesized from substituted propiolactones, but this process will never be competitive with bacterial fermentation since lactone monomers are very costly [152]. Furthermore, chemical synthesis results in polymers with lower molecular weights in comparison to the ones obtained by microbial synthesis [153]. On the other hand, PHA synthesis using genetically modified plants is a process that still needs to deal with several limitations, namely control of monomer composition, plant transformation processes, expression of the transformed genes in the following

generations and the complex extraction of the polymer from the intracellular compartments [154].

Consequently, the current tendency in research is to develop processes carried out by MMC processes. PHA production by MMC follows natural selection principles since microorganisms with PHA storage ability compete with those microorganisms that do not accumulate PHAs. MMC based processes can be carried out in non-sterile conditions and can use organic waste as a carbon source since MMC are more adaptable. Thus, these systems are considered more robust than pure culture based processes, and simultaneously, more cost-efficient [146, 151]. As an example, Crutchik et al. [150] estimated that the minimum PHA cost obtained from sewage sludge were 1.26 and 2.26 US$ kg^{-1} PHA for large and small WWTPs, respectively. These values are quite similar to the fossil-fuel derived plastics cost (1.2 US$ kg^{-1} plastic) and considerably lower to cost the PHA obtained by using pure cultures (between 2.4 and 5.5 US$ kg^{-1} PHA) [150]. Furthermore, MMC based processes can produce a wide range of copolymers with a different composition depending on the feedstock used [151]. Since MMC contain a high variety of bacteria, different pathways can be followed. Copolymers based on 3HB synthesized by MMC can also contain monomers of 3-hydroxyvalerate (3HV), 3-hydroxyhexanoate (3HHx), 3-hydroxy-2-methylvalerate (3H2MV) and 3-hydroxy-2-methylbutyrate (3H2MB) [155]. Therefore, in MMC based processes, one of the easiest ways to modify the final properties is by changing the carbon source used as substrate. In the literature, up to four natural PHA synthetic routes starting from different carbon sources have been described (Fig. 2). Another ten engineered pathways have also been developed [156] but in this review, engineered pathways were not considered for PHA production by MMC.

Pathway I starts with the conversion of a sugar to acetyl-CoA by glycolysis and proceeds with the formation of acetoacetyl-CoA from the condensation of two acetyl-CoA molecules by β-ketothiolase (PhaA),
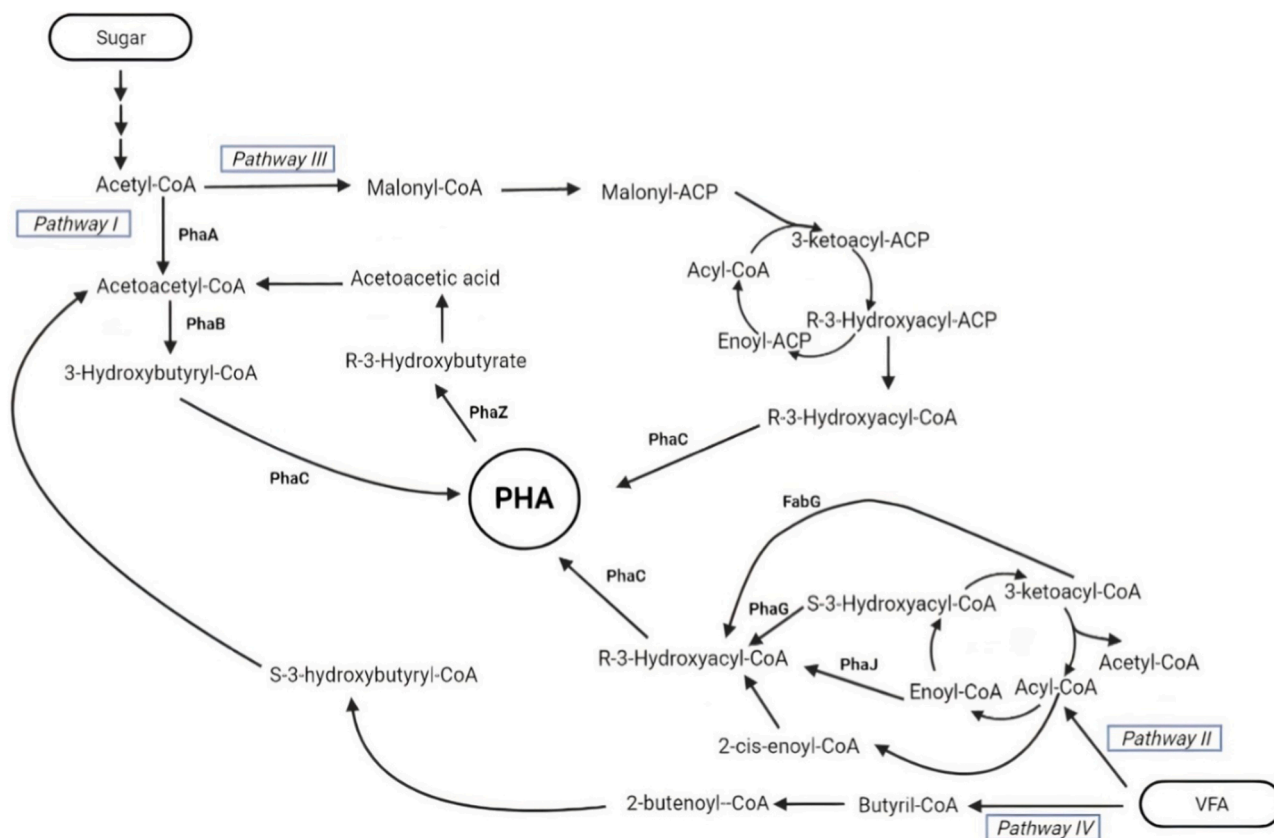

Fig. 2. Metabolic pathways for PHA synthesis.
(adapted from Meng et al. [156]).

which is then transformed into 3-hydroxybutyryl-CoA by NADPH-dependent reductase (PhaB). Finally, the polymerization of 3-hydroxy-butyryl-CoA by PHA synthase (PhaC) leads to the formation of P(3HB) monomers. This pathway is the most well-known and is typically used by *Ralstonia eutrophia* [157]. Concurrently, PHA depolymerase (PhaZ) catalyzes PHA degradation to form 3-hydroxybutyrate, 3-hydroxybutyrate is transformed to acetoacetic acid by 3-hydroxybutyrate dehydrogenase and acetoacetyl-CoA is regenerated from acetoacetic acid by acetoacetyl-CoA synthetase [153].

Pathway II uses fatty acids as substrates, which are converted to acyl-CoA, 3-ketoacyl-CoA, enoyl-CoA and S-3-hydroxyacyl-CoA by the β-oxidation cycle. These intermediates are used to synthesize R-3-hydroxyacyl-CoA by the action of acyl-CoA oxidase and enoyl-CoA hydratase, 3-ketoacyl-CoA reductase (FabG), (R)-enoyl-CoA hydratase/enoyl-CoA hydratase I (PhaJ) and epimerase, respectively. Polymerization of R-3-hydroxyacyl-CoA leads to mcl-PHAs by specific PhaZ [151, 158].

Pathway III starts from the commonly acknowledged 'unrelated' carbon sources, which can be defined as carbon sources with a molecular structure completely different to the resultant PHA monomers [158]. Some examples of unrelated carbon sources are glucose, fructose, glycerol, gluconate, ethanol, and acetate [159]. Through this pathway, also named *de novo* fatty acid synthesis, carbon sources are firstly transformed into acetyl-CoA, acetyl-CoA into malonyl-CoA, and malonyl-CoA into malonyl-ACP. Malonyl-ACP is converted to R-3-hydroxyacyl-ACP, which is then transformed into R-3-hydroxyacyl-CoA by 3-Hydroxyacyl-ACP-CoA transferase (PhaG). Finally, R-3-hydroxyacyl-CoA polymerization gives rise to mcl-PHAs. Both Pathways II and III have been described for most of the *Pseudomonas* spp. [157].

Pathway IV is specific for butyric acid, which could be substrate in PHA synthesis without entering the β-oxidation cycle. Butyric acid is transformed into S-3-hydroxybutyryl-CoA, which is then converted into acetoacetyl-CoA that would follow the same steps of Pathway I to produce P(3HB) monomers. This fourth pathway was observed in Rhizobium (Cicer) sp. Strain CC 1192 [153].

Traditionally, the most widely used substrate in PHA synthesis with pure cultures has been glucose (Pathway I) [146]. However, PHA production by a MMC based process usually starts from VFAs (Pathway II

and IV). On the one hand, VFAs are preferred with respect to other substrates, such as glycerol or carbohydrates, since the last two have the tendency to synthetize glycogen instead of PHAs [160]. Moreover, the synthesis starting from VFAs is energetically more favorable because their complete β-oxidation produces more energy in the form of ATP molecules than the oxidation of a molar equivalent of glucose [161].

However, when using bioproduced VFAs as substrate, the properties and monomer composition of the resultant PHAs would depend on the composition of the VFA-rich-stream [162–164] used as raw material. It has been demonstrated that when using VFAs with an even number of carbons, polymers rich in 3HB monomers are formed. On the contrary, feeding VFAs with an odd number of carbons lead to 3HV monomers [165]. In this sense, the odd-to-even ratio is defined as the sum of odd-equivalent carboxylic acids, such as propionic or valeric acids, divided by the sum of even-equivalent carboxylic acids, such as acetic or butyric acids [60] and it can be used as a parameter to evaluate the ratio between 3HB and 3HV monomeric units present in the final PHA product. Pure polyhydroxybutyrate (PHB) is brittle and stiff, but the incorporation of 3HV in the polymer (as in PHB-co-PHV) enhances elasticity and flexibility [166]. Thus, high odd-to-even ratios are desired in order to enhance polymer mechanical properties [60]. Therefore, odd-equivalent acids must be the main components of the VFA stream feed to the PHA accumulation reactor. Table 5 sums the composition and properties of the PHAs obtained when using different VFAs or mixtures of VFAs in MMC based processes. Since the polymerization and crystallization have a great influence on the final properties of PHAs and they take place inside the cell cytoplasm, the properties of the PHAs produced by MMC based processes are not necessarily the same as the properties of PHAs produced by pure cultures [151].

Furthermore, the cost of the carbon source for PHA biosynthesis can reach 50% of the final production cost [147], so the use of waste-derived VFAs would clearly reduce the price of the final product and make it competitive with fossil-fuel-derived polymers. Also, it would be possible to integrate PHA production and wastewater or organic waste treatments [160]. In a MMC based process using waste or wastewater as substrate, PHA synthesis takes place in four steps:

Table 5

Thermal and mechanical properties of PHAs produced by MMC cultures. T_m, melting temperature; ΔH_m, melting enthalpy; T_g, glass transition temperature; PDI, polydispersity index; M_w, weight average molecular weight.

Substrate	VFA composition (% mol) (HAc:HPr:HBt:HV:other)	PHA composition (% mol) (3HB:3H2MB:3HV:3H2MV:3HHx)	PHA content (% gPHA g^{-1} VSS)	Thermal properties					Molecular weight		Reference
				$T_{m(1)}$ (ºC)	$T_{m(2)}$ (ºC)	Total ΔH_m (J g^{-1})	T_{g1} (ºC)	T_{g2} (ºC)	PDI	M_w (x 10^5)	
Acetate	100:0:0:0:0	90:4:4:1:1	–	171	–	77	5	–	2.0	8.1	[155]
Acetate	100:0:0:0:0	66:4:28:2:0	–	96/109	146/161	9	-6	–	1.4	5.5	[171]
Acetate	100:0:0:0:0	100:0:0:0:0	21	171	–	64	–	–	1.3	32	[172]
Acetate	100:0:0:0:0	96:0:4:0:0	52	–	–	–	–	–	2.2	0.9	[173]
Acetate	100:0:0:0:0	NA	26	93	150	32	-6	–	2.3	2.2	[174]
Propionate	0:100:0:0:0	12:6:63:14:6	–	89	–	18	-14	–	2.0	4.5	[155]
Propionate	0:100:0:0:0	11:13:35:41:0	–	84	20	–	-0.2	–	1.5	5.6	[171]
Propionate	0:100:0:0:0	NA	30	92	38	104	-18	–	2.8	4.3	[174]
Butyrate	0:0:100:0:0	83:5:7:2:2	–	137	150	50	3	–	1.7	9.0	[155]
Valerate	0:0:0:100:0	12:5:78:4:1	–	98	–	2	-12	–	3.9	6.2	[155]
Acetate + propionate	82:18:0:0:0	55:5:30:9:0	–	124	156	632	– 8	–	3.1	3.9	[171]
Acetate + propionate	54:47:0:0:0	46:8:32:15:0	–	70	97	19	-2	–	1.7	5.0	[171]
Acetate + propionate	22:78:0:0:0	20:9:39:32:0	–	119	155	10	-6	–	1.4	5.5	[171]
Acetate + propionate	60:40:0:0:0	94:0:6:0:0	25	157	–	54	1	–	1.3	33	[172]
Acetate + propionate	84:16:0:0:0	60.3:0:39.7:0:0	54	–	–	–	–	–	3.2	1.4	[173]
Acetate + propionate	64:36:0:0:0	NA	32	82	24	93	-19	–	1.7	2.3	[174]
VFA mix	47:18:22:13	61:0:39:0:0	77	113	138	–	-16	–	2.3	2.1	[164]
VFA mix	73:13:12:0.02	79:0:21:0:0	68	121	137	–	-10	–	2.7	3.9	[164]
VFA mix	74:7:16:3	85:0:15:0:0	56	134	147	–	-1	–	2.3	6.5	[164]

*NA: not available

1) Acidogenic fermentation of the waste material to produce a VFA-rich stream.
2) Enrichment of aerobic activated sludge by feast-famine feeding strategies that would select the PHA-accumulating populations.
3) Improvement of the accumulation of PHAs in the enriched biomass.
4) Extraction of the PHAs from the biomass.

Factors influencing PHA production in the enrichment and accumulation steps have been previously reviewed [160,167]. Also, extraction methods for PHA recovery have been extensively evaluated [148, 168]. For an industrial application, it would be necessary to produce PHAs with a fixed composition to maintain the quality and properties of the bioplastics. It has been demonstrated that PHA composition can be controlled by shifting the VFA composition in the feeding stream [155, 169,170]. Therefore, to obtain a PHA with a constant composition over time, the VFA composition of the feeding stream must also be constant. Then, for developing a commercial PHA production from organic wastes, more knowledge is needed in the control of VFA composition obtained in the acidogenesis fermentation. Furthermore, in both PHA enrichment and accumulation steps, the VFA concentration in the VFA-rich stream used as substrate is also a key parameter.

6. Conclusions and research gaps to be solved

Volatile fatty acids are value added products and their synthesis from organic waste or wastewaters by MMC based processes is a promising technology that would avoid the use of fossil fuels. However, further knowledge about how to control VFA production yield and composition is needed to scale up bioprocesses based on the use of waste streams as feedstock. This review rounds up the main metabolic routes that lead to VFA synthesis by MMC. Next, the interactional effect of pH, temperature and OLRv and HRT and the organic composition of the waste on VFA production yield is discussed. From the aforementioned analysis, it can be gathered that organic composition of waste, in terms of carbohydrates, proteins and lipids, is the main factor that affects the VFA production yield, even though the operational parameters, like pH,

temperature and OLRv and HRT also play a role. In this sense, Table 6 sums up the operational conditions that lead to a higher VFA production yield depending on the organic composition of the waste. Likewise, the effect of pH, temperature and bacterial composition on VFA composition was analyzed together with substrate composition. The effect of the aforementioned factors on VFA composition might be interconnected and is still not clear. More research is needed to predict and control VFA composition of the effluent of an acidogenic fermentation reactor. On this matter, energy-based metabolic models are presented as the best tool known to date to predict VFA composition from a substrate with a well-characterized composition under different pH conditions. Finally, the PHA synthesis process by MMC based processes was presented as an example of one of the most interesting applications of the waste-based bioproduced VFAs.

Despite all the research performed in the last years in the VFA fermentation platform, there is still limited knowledge in some areas that should be studied deeply. Further work needs to cover the following gaps:

- Establishment of standard parameters to quantify both VFA production yield and VFA composition. In this review, studies of VFA fermentation of solid, slurry-like, and liquid wastes (mostly wastewaters) have been analyzed together. Nevertheless, the VFA production yields are expressed differently, depending on the type of waste (solid and/or liquid waste) research field. Moreover, some studies examine the operational parameters that maximize VFA production, quantifying it as the degree of acidification or the VFA concentration obtained instead of the VFA production yield. Additionally, VFA compositions are sometimes related to the sum of all the VFAs obtained and on other occasions, related to the total soluble organic matter. The use of standard parameters would facilitate the comparison between different studies.
- Further study of the VFA composition obtained from different substrates regarding their organic components rather than considering it only as a concrete type of substrate. As emphasized in this review, organic composition of the substrate, in terms of carbohydrates, proteins and lipids, has a strong influence on both VFA production yield and the type of VFA produced. Additionally, attention should be paid when reviewing literature since substrates with different organic compositions and complexities have been grouped under the same substrate name, even when its composition, origin and seasonality is different. Also, the substrate consumption needs to be specifically analyzed in terms of carbohydrate, protein and lipid amounts apart from soluble COD amounts.
- Further analysis of the microbial communities to link the different species to the different VFAs obtained. There are still many unidentified species among the acidogenic bacteria. Systematic studies of microbial communities in an acidogenic reactor will help to better know the bacteria responsible for the production of each VFA as well as their metabolic interactions.
- Study of the specific organic loading rate (OLRs) as another parameter that can affect both VFA production yield and VFA composition. Unless in some studies of solid waste fermentation, where the parameter inoculum-to-substrate ratio is regarded, the amount of inoculum present in the reactor is rarely considered. We found little data that allow us to analyze this parameter. On some occasions, authors only detail the origin of the inoculum, but they do not specify the amount added or its concentration in the acidogenic fermentation reactor. We consider that the amount of available substrate for a certain amount of biomass might affect the VFA yield or composition. OLRs is a parameter that could serve to analyze this effect.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

Table 6
Operational conditions that lead to high VFA production yields depending on the organic composition of the substrate.

Operational conditions that lead to high VFA production yields				
Parameter		Main component of the substrate		
		Carbohydrates	Proteins	Lipids
pH	*Solid waste*	Alkaline pH	Alkaline pH	Alkaline pH
	Slurry-like waste	Complex carbohydrates: alkaline pH Simple carbohydrates: neutral pH	Alkaline pH	Alkaline pH
	Liquid waste	Complex carbohydrates: alkaline pH Simple carbohydrates: neutral pH	Complex proteins: alkaline pH Simple proteins: neutral pH	Mesophilic conditions: alkaline pH Thermophilic conditions: neutral pH
Temperature	*Solid or slurry-like waste*	Mesophilic conditions	Thermophilic conditions	ND
	Liquid waste	More research is needed	More research is needed	Mesophilic conditions
OLRv	*Solid, slurry-like or liquid waste*	Simple substrates: high OLRv to inhibit methanogenesis Complex substrates: maximum OLRv restricted by hydrolysis		

the work reported in this paper.

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