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DARK FERMENTATIVE VOLATILE FATTY ACIDS PRODUCTION FROM FOOD WASTE - A REVIEW OF THE POTENTIAL CENTRAL ROLE IN WASTE BIOREFINERIES

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Abstract

Volatile fatty acids (VFAs) are high-value chemicals that are increasingly demanded worldwide. Biological production via food waste (FW) dark

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fermentation (DF) is a promising option to achieve the sustainability and environmental benefits typical of biobased chemicals and concurrently manage large amounts of residues. DF has a great potential to play a central role in waste biorefineries due to its ability to hydrolyse and convert complex organic substrates into VFAs that can be used as building blocks for bioproducts, chemicals, fuels. Several challenges must be faced for full-scale implementation, including process optimization to achieve high and stable yields, the development of efficient techniques for selective recovery, and the cost-effectiveness of the whole process. This review aims to critically discuss and statistically analyse the existing relationships between process performance and the main variables of concern. Moreover, opportunities, current challenges, and perspectives of a FW-based and fermentation-centred biorefinery layout are discussed.

Keywords: acidogenic fermentation; waste derived VFAs; bioenergy; biobased products; integrated bioprocesses

1. INTRODUCTION

Approximately a third of the food globally produced for human consumption (1.3 billion tons of wasted food, either edible or non-edible) is lost every year throughout the supply chain, from agricultural production to final household consumption (Sharma et al., 2020). In the European Union (EU), about 88 million tonnes of food (an estimated 20% of the whole production) are wasted every year, equivalent to about 173 kg per capita in the EU-28, according to the most recent data published by Eurostat. In the absence of adequate prevention and minimization measures, these figures are expected to increase, especially if the global population and food overproduction continue to expand.

In the present work we refer to the food waste (FW) that is produced at the end of the food supply chain, which typically occurs in the retail and consumption stages and does not include residues from the agri-food system. The management of increasingly large amounts represents a serious issue from an economic, social, and ethical point of view. Valuable resources are wasted, often asymmetrically with respect to the needs to be met. Waste avoidance represents the optimal solution for FW, more than for any other type of waste, as it also has an obvious strong ethical significance. However, although initiatives are multiplying related to the optimization of food production, transport and sales, as well as to increase the awareness of consumers towards unnecessary purchasing, the production of nonedible residues is inevitable along the entire chain (Teigiserova et al., 2019). FW production must be adequately balanced by controlling potential impacts and, from a circular economy perspective, by optimizing resource recovery both quantitatively and qualitatively to approximate the concept of zero waste and to make recovered products attractive on the market, respectively. These objectives can be achieved through an appropriate integration of processes to pre-treat, simplify, and convert the residual organic matter. In this respect, the concept of waste biorefinery represents the technical solution, as well as an even more sustainable evolution of the original biorefinery concept and, lastly, the link between bioeconomy and circular economy promoted by EU policies (Dahiya et al., 2018; Moretto et al., 2020; Patel et al., 2019; Strazzera et al., 2018). Waste biorefineries may represent the transition toward more innovative strategies for FW valorisation, beyond traditional options such as biogas/biomethane production and composting, aiming at converting organic substrates into high-value products or building blocks. Among recoverable products, volatile fatty acids (VFAs) may be attractive because of increasing market demand. VFAs are found to be used in a wide range of applications such as food and beverage, animal feed, pharmaceuticals, personal care and cosmetics, lubricants, and agriculture. The global market demand is expected to grow at a CAGR (Compound Annual Growth Rate) of 4.6% over the 2019-2025 period and to reach a value of 9.9 billion € by 2025 (Market Research Report, 2021). Among VFAs, propionic acid has the highest market price (2.0-2.5 € kg^{-1}), followed by butyric and caproic acids (1.5-1.6 € kg^{-1}) and acetic acid (0.4-0.8 € kg^{-1}) (Atasoy et al., 2018); acetic acid will arguably dominate global demand as it finds wide applications in food storage and packaging industry (Atasoy et al., 2018). Currently, commercial production of VFAs mostly relies on chemical routes through oxidation or carboxylation of chemical precursors from petroleum processing, but low-cost biological production is seen as a sustainable alternative. VFAs can be synthesized from organic waste streams, even in a heterogeneous mixture, by mixed microbial cultures (MMC) performing hydrolysis and acidogenic fermentation processes such as dark fermentation (DF) (Bastidas-Oyanedel et al., 2015; Garcia-Aguirre et al., 2017; Garcia et al., 2018; Moretto et al., 2019). The use of MMC removes the need for preliminary biomass selection (as otherwise required in biorefineries that target a single product), thus reducing operating costs, facilitating process control, and, in turn, making the production of waste-derived VFAs potentially feasible. From a qualitative point of view, the high content of carbohydrates makes a residue potentially attractive for the production of carboxylic acids (Chen et al., 2013a; Yin et al., 2016). Food waste is, besides proteins- and lipids-, a carbohydrate-rich source that can be converted to gaseous and soluble bioproducts (Alibardi and Cossu, 2016); this figure, together with the wide availability and the continuous amounts generated, makes FW an attractive substrate for biorefineries (Alibardi et al., 2020; Dahiya et al., 2018; Moretto et al., 2020; Strazzera et al., 2018; Zhou et al., 2018).

The present review aims at discussing the central role of DF and VFA production in a FW-based biorefinery. Although various reviews on the production of VFAs have been published, they have typically focused on a range of different substrates (among the others: Atasoy et al., 2018; Lee et al., 2014; Sekoai et al., 2021); this complicates data analysis and the identification of the optimal operating conditions. We have specifically oriented this survey to FW, so to build a thorough database of the available data and critically analyse the influence of the main parameters that govern the process. More than 170 related studies, mostly published during the last years, were consulted (Scopus search, keywords used: "biorefinery", "dark fermentation", "acidogenic fermentation", "volatile fatty acids" and "food waste") and screened for reliability and consistency. Information on operating conditions was provided and processed using statistical methods to identify the optimal region for VFA production. In Chapters 6 and 7, the VFAs separation processes and the most interesting applications are reported to make the reading more comprehensive. The discussion culminates the proposal of a layout for a fermentation-centred FW biorefinery and of the related opportunities, current challenges, and perspectives.

2. DARK FERMENTATION - MAIN METABOLIC PATHWAYS

Under appropriate conditions, microorganisms can convert organic substrates into gaseous products, mainly H_2 and CO_2, and a mix of VFAs and reduced end products, including alcohols (De Gioannis et al., 2017). Dark fermentation of organic substrates has been widely studied, mainly with a focus on H_2 production (Dong et al., 2009; Nathao et al., 2013), while fewer studies have specifically targeted VFA production. In fact, although the volumetric production of H_2 from FW can reach values of 1.7–5.6 N l H_2 $l_{reactor}^{-1}$ (De Gioannis et al., 2013), the H_2 generated represents no more than 3-4% w w^{-1} of the total substrate mass consumed, while VFAs account for ~65% w w^{-1} of the degraded organic matter (Bastidas-Oyanedel et al., 2015). It would therefore make sense if VFAs, as well as H_2, were the target fermentation products, from an integrated fermentation-centred biorefinery perspective (Atasoy et al., 2018; Dahiya et al., 2015; Strazzera et al., 2018). The recovered H_2 could be separated from the CO_2 and used as a stand-alone energy carrier or mixed with CH_4 to obtain the gaseous fuel known as hythane (Roy and Das, 2016), or as a chemical for CO_2 reduction to produce biomethane through the hydrogenotrophic pathway that would be compatible with the recovery of VFAs (Aryal et al., 2018). However, the production of organic acids presents additional challenges because the range of soluble products is much broader than that of gaseous ones, and complex separation and purification are required in view of commercial use (Arslan et al., 2016). Fermentation of a complex substrate such as FW involves the spontaneous onset of multiple metabolic pathways, especially if the process relies on autochthonous microbial consortia, resulting in the generation of a wide range of products, including acetate, propionate, butyrate, ethanol, H_2, and CO_2 (Zhou et al., 2018). The type of prevailing pathway depends on the fermentation conditions (mainly temperature and pH), but also on substrate composition and nature of the involved microbial consortia. The main metabolic pathways during DF can be summarized as acetate and butyrate-type, propionate-type, mixed-acid, acetate-ethanol type, lactate-type and homoacetogenic fermentation routes, as reported in Table 1 (Equations 1-8).

Table 1 here

As shown in Figure 1, pyruvate is a branch point intermediate that can be converted to acetyl-coenzyme A (CoA) leading to the formation of acetate and butyrate through two analogous pathways (Chen et al., 2013a).

Figure 1 here

Equations (1) and (2) summarise the stoichiometric relationships between the fermentable sugars (glucose) generated from carbohydrates by hydrolytic bacteria and acetate and butyrate as fermentation products. The chemical reactions are catalysed by acid-forming enzymes taking part in short-chain fatty acids (SCFAs) production. More in detail, four enzymes play critical roles in the production of acetic and butyric acids (Zhu and Yang, 2004): acetyl-CoA and butyryl-CoA are first converted to acetyl phosphate and butyryl phosphate by phosphotransacetylase (PTA) and phosphotransbutyrylase (PTB), which are further converted to acetate and butyrate by acetate kinase (AK) and butyrate kinase (BK), respectively. Acetate can be produced not only from pyruvate through the acetyl-CoA pathway, but also from the oxidation of ethanol or longer chain fatty acids (C3 and above) through the action of syntrophic bacteria (H_2-producing acetogenic bacteria). Indeed, although ethanol could be produced during fermentation of organic materials according to Eq. (5), with acetaldehyde as intermediate, it is not considered a common DF product of FW (Zhou et al., 2018). Acetate can also be produced by a group of obligate anaerobe bacteria called homoacetogens that use H_2 as an electron donor to reduce CO_2 to acetate according to the homoacetogenic fermentation pathway (Eq. (6)) (CataSaady, 2013).

Propionate could be produced through two distinct pathways: from reduction of pyruvate by propionate dehydrogenase with lactate as the intermediate (Lee et al., 2008), or through carboxylation of pyruvate to form oxaloacetate then reduced to propionate through malate, fumarate, and succinate, with succinyl-CoA, methylmalonyl-CoA, and propionyl-CoA as intermediates (Ciani et al., 2008).

When propionate-type fermentation is dominant, one mole of glucose could theoretically generate two moles of propionate (Eq. (3)), but anaerobic microorganisms commonly ferment glucose to propionate along with acetate and CO_2 (Zhu et al., 2009).

It is worth pointing out that H_2 is always the accompanying product in the acetate and butyrate-type metabolic pathway, whilst the propionate-type is a neutral or H_2-consuming fermentation pathway, and homoacetogenesis consumes H_2. Regarding the lactate-type metabolic pathway, two key enzymes are involved: lactate dehydrogenase (LDH), which produces lactate from pyruvate, and NAD-independent LDH (iLDH), which is responsible for producing pyruvate from lactate. The lactate-producing process can be divided into two fermentation types: i) homolactic fermentation, according to which 2 mol of pyruvate are produced from the glycolysis of glucose and then reduced to two moles of lactic acid (Eq. (7)) and ii) heterolactic fermentation, in which one mole of lactic acid is produced along with CO_2 and ethanol (or acetate) (Eq. (8)) (Castillo Martinez et al., 2013). Numerous studies reported high VFA yields and concentrations ranging from 0.04 g L^{-1} to 41 g_{COD} L^{-1} achievable through FW dark fermentation (Fig. 3). Although VFA mixtures are typically obtained, selective VFA production might be achieved by promoting specific metabolic routes. In this respect, different yields and relative proportions between VFAs are achievable by properly setting the main operating parameters such as pH, temperature, hydraulic retention time (HRT) and organic loading rate (OLR) (De Gioannis et al., 2013b; Feng et al., 2011; Jiang et al., 2013; Wang et al., 2014). However, relatively little information is available on the influence of such parameters on VFA production, as most literature studies targeted H_2 rather than VFA production, and further variables must be considered such as substrate composition, presence of co-substrates, type of inoculum and applied pre-treatment, reactor type and mode of operation. DF is a complex process, especially when performed on complex substrates that carry native microorganism populations. Due to the intricate interrelations among the above-mentioned factors, the optimization of the process requires a deep understanding of the metabolic pathways and the effects of the main factors and operating parameters for maximizing VFA production.

3. ORIENTING DARK FERMENTATION TOWARDS VFA PRODUCTION – CRITICAL FACTORS

3.1 Substrate composition

FW is essentially composed by three groups of macromolecules (carbohydrates, proteins and lipids), which can influence both the amount and the chemical composition of the VFAs produced through DF (Strazzera et al., 2018). Carbohydrates are easily hydrolysed into monomeric sugars that can be readily fermented to VFAs (De Gioannis et al., 2013b; Shen et al., 2017). The use of more concentrated carbohydrate-rich substrates has been reported to increase total acid production in neutral pH ranges (Arslan et al., 2016). Proteins may enhance the fermentation process by providing nutrients for microbial growth. However, FW protein hydrolysis is considered a rate limiting step during acidogenic fermentation (Shen et al., 2017) and, as previously observed by Feng et al. (2009) and Shen et al. (2014), the production of VFAs from protein-rich substrates is lower compared to carbohydrates, due to inhibition of microbial activity caused by the accumulation of free ammonium. Lipids, whose hydrolysis produces glycerol and long-chain fatty acids (LCFA), are less prone to fermentation than carbohydrates, because of lower solubility and slower biodegradation kinetics and represent the substrate of major concern during the acidogenic reactions. In fact, as reported by Dong et al. (2009), LCFAs can adhere to the cellular wall, affecting the transport of nutrients, and, consequentially, inhibiting the metabolism of bacteria. Concerning the final distribution of fermentation products, it is generally reported that the degradation of carbohydrate-rich substrates leads mainly to the production of acetic, butyric, and propionic acids (Cappai et al., 2014; Alibardi and Cossu, 2016; Arslan et al., 2016; Yin et al., 2016), while the production of valeric and isovaleric acids is supported by protein-rich substrates such as meat and bone meal (Garcia-Aguirre et al., 2017; Shen et al., 2014). Although a clear influence of the substrate type on the final product composition has been recognized, it is difficult to establish a clear correlation. Alibardi and Cossu (2016) found that carbohydrate content was the main factor that influenced butyrate production, which was found to be comparable to acetate, with a butyrate-to-acetate-ratio > 0.8. Shen et al. (2017) investigated two types of protein-rich substrates (tofu and egg white) as a source of VFAs and found that valeric acid represented 18-25% of total VFA produced, being the second highest after acetic acid. The correlation between a reduction in the carbon/nitrogen (C/N) ratio and a metabolic shift from VFA production to solvent production (e.g. ethanol) was observed by Lin and Lay (2004). Few studies have been reported in the literature on lipid-rich substrates. Propionic acid production appears to be mainly supported by glycerol-rich waste streams, but the results are controversial and probably influenced by the operating conditions adopted (Shen et al., 2014; Silva et al., 2013). In this regard, Jankowska et al. (2017) observed that different substrates lead to a similar spectrum of products in MMC and stated that the substrate characteristics barely influence the distribution of VFAs compared to other process parameters such as pH. The large variability of data reported in the scientific literature in terms of VFA concentration and distribution is likely to depend also on the complexity, heterogeneity, geographical and seasonal variability of FW composition (Feng et al., 2009). This implies that the combined effect of substrate characteristics and operating conditions must be systematically investigated to identify optimal conditions that maximize production yield and orient the VFA distribution (Atasoy et al., 2018; Lee et al., 2014).

3.2 Inoculum source

Sewage activated sludge from municipal wastewater treatment plants are widely used as inocula for dark fermentation of FW (Chen et al., 2013a; Cappai et al., 2014; Feng et al., 2011; Wu et al., 2016) and anaerobic sludge (Arras et al., 2019; Dahiya et al., 2015; Garcia-Aguirre et al., 2017; He et al., 2019; Jiang et al., 2013; Wang et al., 2014; Yin et al., 2014). As mentioned previously, the use of MMC would be more advantageous on the industrial scale than pure cultures: as sterilization would not be required, a wider range of complex substrates could be treated due to a higher diversity of enzymes (Deng and Wang, 2016), and overall process costs would be reduced (Bastidas-Oyanedel et al., 2015). The bacteria most commonly involved in DF are the obligate anaerobes of *Clostridium sp.*, effective in converting a wide range of carbohydrates with high H_2 and organic acid yields, or the facultative anaerobes of *Escherichia coli* and *Enterobactericeae sp.,* although characterized by a lower H_2 yield (O-Thong et al., 2018). To enhance VFA production, fermentative bacteria should be selected from the inoculum and the activity of methanogens suppressed by appropriately adjusting operating parameters such as pH and hydraulic retention time (as better described in Sect. 3.4), applying thermal or pH shock pre-treatment (Cappai et al., 2014; Lin and Lay, 2004) or using chemical inhibitors of methanogenesis (Liu et al., 2011). However, inoculum pre-treatments could affect the economic viability of the process and require careful consideration. While the use of pure cultures and homogeneous/selected substrates makes the industrial production of specific acids possible (Chen et al., 2013b; Yan et al., 2014), the goal is much more difficult in the case of MMC and heterogeneous residual substrates such as FW, where several organisms are simultaneously competing for a complex substrate. It is no coincidence that this aspect is considered one of the most difficult issues to sort (Arslan et al., 2016). Wang et al. (2014) evaluated the effects of different MMC on VFA production from FW, adopting various operating pH (4, 5, 6, and no control) in batch tests. The anaerobic activated sludge performed better than the aerobic in terms of the yield of VFAs (0.92 vs 0.48 g_{VFA} g_{VSS}^{-1}), and the distribution of VFAs was 70% butyric, 17% acetic and 5% propionic acid. Yin et al. (2016) obtained a VFA yield of 0.79 g_{COD} g_{VS}^{-1} during acidogenic fermentation of FW using anaerobic sludge and under limited aeration conditions (ORP – 100, – 200 mV). Arras et al. (2019) studied the influence of three types of anaerobic cultures on the hydrolysis and acidogenesis of FW; the inoculum were sourced from different treatment plants and sections operated at different temperatures. The results obtained showed that the origin of the inoculum has more marked effects on the evolution of the acidogenic phase than on the hydrolysis of the substrate; the inoculum from wastewater and food waste treatment sections showed promising conversion efficiencies (VFAs = 60-70% of the solubilized organic substance).

3.3 Reactor configuration and operation mode

The configuration of the reactor influences the hydrodynamics and, therefore, the substrate-microorganism contact and the liquid-gas mass transfer. The overhead gas pressure can lead to inhibitory effects, as a high H_2 partial pressure proved to favor the production of reduced compounds such as lactate, ethanol, and propionate, which is associated with zero hydrogen production or even consumption (Zhou et al., 2018). Suspended and attached growth are common conditions used in the fermentation production of VFAs and have led to the development of different types of bioreactors (Khan et al., 2016; Lee et al., 2014). Although most of the bioreactors used for solid-state dark fermentation are of the CSTR type (continuously stirred tank reactor) (Cappai et al., 2014; Dahiya et al., 2015; He et al., 2019; Jiang et al., 2013; Shin et al., 2004), adopting attached growth technologies may prevent biomass washout and guarantee a higher biomass concentration in the reactor. Anaerobic leach bed reactors, UASB (up-flow anaerobic sludge blanket) and ASBR (anaerobic sequencing batch reactor) reactors have been proposed too (Xu et al., 2012; Zhang et al., 2008). However, clogging of the packing material may be an issue (Khan et al., 2016), especially when wastes containing high concentrations of suspended solids are treated. In addition, reduced mixing limits mass transfer, resulting in lower substrate conversion and gas accumulation in the biofilm with consequent inhibitory effects. Although the adoption of a longer solid retention time (SRT) can increase VFA production, it can also favor slow-growing methanogens that result in depletion of organic acids (Lee et al., 2014). Regarding the operation mode, batch, fed-batch, semi-continuous, and continuous modes can be adopted. According to Lee et al. (2014), the continuous mode might not be feasible for slow reactions, whilst the batch or semi-continuous operation mode seems to be more favourable for VFA production, especially in the case of UASB, packed and fluidized bed reactors.

3.4 Operating parameters

3.4.1 pH

Among the parameters that govern the production of fermentative FW VFAs, pH is the most studied and influencing one but also, as clearly appears from the data below, highly controversial. The range suitable for VFA production falls within the range 5 – 7, since enzymatic hydrolysis of FW has an optimum at pH 6 (Wang et al., 2014). Ren et al. (2011) observed that the activity of acidogenic bacteria would be largely reduced at pH below 4, whilst pHs higher than 6.5 could favor the transition to methanogenesis (Yuan et al., 2006). Several authors reported that a weakly acidic pH should be maintained to achieve significant VFA production and enhance production kinetics (Jiang et al., 2013; Lim et al., 2008). Lim et al. (2008) obtained a total concentration of VFAs of 25 g L^{-1} and a yield of 0.37 g_{VFA} g^{-1} of VS_{fed} applying a pH of 5.5 at 35 ° C, while at the same pH value of 5.5, Garcia et al. (2018) observed a maximum concentration of VFAs of 30 g L^{-1} during semi-continuous fermentation of FW. Jiang et al. (2013) observed a maximum VFA concentration of 39.5 g L^{-1} and a maximum yield of 0.32 g_{VFA} g^{-1} of VS_{fed} when controlling pH at 6. Wang et al. (2014) obtained a concentration of VFAs of 32.4 g L^{-1} at pH 6 using activated anaerobic sludge as inoculum. Cappai et al. (2014) performed several tests on a mixture of FW (45%wt) and heat shock activated waste sludge (55%wt) using different operating pH values at 39°C; the highest VFA concentration (13 g L^{-1}) was obtained at pH 6.5. As reported by Chang et al. (2010), a total VFA concentration of 34.6 g L^{-1} and a yield of 0.49 g_{VFA} g^{-1} of VS_{fed} was obtained applying a pH of 7 at 40°C; Zhao et al. (2006) achieved a similar VFA concentration (36 g L^{-1}) at pH 7, while a decrease of 25.7, 24.3 and 28.5 g L^{-1} was observed at pH 5, 9, and 11, respectively, although VFA production remained higher than when no pH control was performed (20.1 g L^{-1}). It is worth noting that a significant VFA production was also achieved under alkaline conditions. High pH is indeed beneficial for the solubilisation and degradation of fats and proteins, and prevents the growth of both hydrogenotrophic and acetoclastic methanogens (Dahiya et al., 2015; Garcia-Aguirre et al., 2017). In this regard, Dahiya et al. (2015) observed a higher VFA production at an initial pH (without subsequent control) of 10 (6.3 g L^{-1}) as compared to pH 9 (5.2 g L^{-1}), pH 6 (4.5 g L^{-1}), pH 5 (4.2 g L^{-1}), pH 7 (4.1 g L^{-1}), pH 8 (3.8 g L^{-1}) and pH 11 (3.5 g L^{-1}). Garcia-Aguirre et al. (2017) conducted a comparative study under mesophilic conditions (35°C) and observed the highest concentration of VFA of 8.3 g_{COD} L^{-1} at an initial pH of 10, compared to approximately 6 g_{COD} L^{-1} at pH 5.5. The operating pH can also affect the type of VFA produced from FW. In general, metabolic pathways involving acetate and butyrate production are favoured in a pH range of 5 – 6, whilst slightly lower pHs would favor the production of butyrate at the expense of acetate (Infantes et al., 2011), and neutral or higher pH up to 8 promote propionate production (Cappai et al., 2014). This general statement is confirmed by several studies. Lim et al. (2008) observed that acetic acid was the main product (49.2%) when implementing an operating pH of 5.5, followed by propionic (23.5%) and butyric acid (20.7%). Jiang et al. (2013) found that acetate and butyrate accounted for more than 90% of total VFA production at pH 5 and approximately 77% at pH 6 and 7; propionate was observed to an appreciable extent (13.5% and 19.7%) at pH 6 and 7. Hawkes et al. (2002) observed the conversion of acetate and butyrate to propionate production as the pH increased. Many experiments have been conducted applying an operating pH value of 6; under this condition, Wang et al. (2014) and Yin et al. (2014) observed a clear prevalence of acetic and butyric acids (>90% of the total VFA production). The prevalence of the production of acetic and butyric acids can be accompanied by a noticeable presence of propionic acid already at slightly higher operating pH values, for example, at pH = 6.5 as reported by Cappai et al. (2014), or at pH = 7 as reported by Chang et al. (2010). However, it is worth noting that other studies have shown different results, highlighting that acetic and butyric acid production from complex substrates such as FW can also be promoted under alkaline conditions, which could be explained by the predominance of phosphoroclastic degradation pathways (Dahiya et al., 2015). Zhao et al. (2006) found that about 71.9% of total VFAs was butyric acid at pH 5, but this figure increased to 73.4% at pH 7, and >45% of total VFAs was acetic acid at pH 9 and 11. Formic acid appeared according to 6.5%, 2.4%, 24.7% and 30.8% at pHs 5, 7, 9, and 11, respectively, while only a small amount of propionic acid was produced under all the conditions studied. These differences in terms of experimental evidence will probably not surprise those who are familiar with FW fermentation. Indeed, when results obtained at a given pH are compared, the influence may be overlooked by differences in substrate, or seed sludge, or operating conditions adopted.

Moreover, the composition of the FW can vary significantly depending on the geographical and social context in which it is produced. To this regard, Lee et al. (2014) appropriately state that the optimal pH for obtaining a specific VFA from FW is highly dependent on the composition of the substrate under concern, while the pH values are often adopted from previous studies performed on FW of different composition or even simple substrates such as glucose. Finally, it is worth underlying that, despite the advantages for VFA production, adjusting pH to weakly acidic or alkaline conditions by adding a large amount of chemicals could raise the production cost and the process complexity.

3.4.2 Temperature

Temperature is a key parameter for acidogenic fermentation of FW, due to its direct involvement in microbial growth, metabolism, and kinetics of microbial processes (Arras et al., 2019; Strazzera et al., 2018). Acidogenic fermentation has been largely studied under mesophilic conditions (25 – 45°C) (Cappai et al., 2014; Jiang et al., 2013; Shin et al., 2004), while few studies have been performed under thermophilic conditions (50 – 60° C) (Garcia-Aguirre et al., 2017; He et al., 2019; Jiang et al., 2013; Komemoto et al., 2009) and even fewer under hyperthermophilic conditions (65 – 75°C) (He et al., 2019; Kim et al., 2006). A temperature increase, while remaining within the mesophilic range, is beneficial in terms of VFA concentration, yield, and production rate. More in detail, higher

VFA production can be achieved by increasing the temperature to 40 – 45°C, considered the optimal temperature for hydrolysis rates and most fermentation reactions (Arslan et al., 2016; Jiang et al., 2013), while a further increase to 55°C has a detrimental effect due to thermal denaturation of proteins and essential enzymes. Garcia-Aguirre et al. (2017), Komemoto et al. (2009) and He et al. (2019) also observed negative effects of temperature values around 55°C under both acidic and alkaline conditions. He et al. (2019) found a decrease in the total VFA concentration from 16.7 g L^{-1} at 35°C to 11 g L^{-1} at 55°C after 7 days of fermentation. A further increase to 70°C led to an even lower VFA concentration of 13.5 g L^{-1}. Since increasing the operating temperature to enhance VFA production requires a careful balance between benefits and operating costs, it is commonly assumed that an efficient and economical value is in the range 35-37°C, while psychrophilic conditions are considered unsuitable for any application. Regarding the type of VFA produced, according to Shin et al. (2004) and Jiang et al. (2013), mesophilic conditions appear to promote the production of acetic and propionic acids, while a metabolic shift from acetate to butyrate is observed at increased operating temperatures (50 – 55°C) (Arras et al., 2019; Hussain et al., 2017).

3.4.3 Hydraulic retention time

The hydraulic retention time (HRT) must be adequate to allow for hydrolysis and acidogenesis whose rate is particularly limiting for heterogeneous and complex solid substrates such as FW. Theoretically, since hydrolysis is commonly recognised as the limiting step of the process, the production of VFAs from FW is expected to increase with HRT. On the other hand, too long HRTs (> 5 – 7 d) would favour methanogens (at pH values > 6.5) and, especially in view of a full-scale implementation, would reduce the mass rate of waste to be treated, requiring larger reactor volumes, and entail higher capital costs. Zhou et al. (2018) showed that too long HRTs may lead to stagnant VFA production due to substrate limitation. This was also reported by Lim et al. (2008) who found that the VFA yield increased by extending the HRT from 4 to 8 d, while no significant benefit was observed when the HRT exceeded 12 d. Garcia-Aguirre et al. (2017) observed that an HRT of 4 d was necessary to achieve 83% of the final VFA production under weakly acidic (pH 5.5) and mesophilic conditions (35°C). The duration of the process also influences the type of acids produced and their possible biochemical transformations. In general, for short HRTs (4-8 d) acetic acid would represent the main fermentation product, followed by propionate and butyrate, while propionate would prevail at longer HRTs (> 12 d), probably due to the higher concentration of H_2 available to microorganisms, followed by acetate (Lim et al., 2008).

The most used type of reactor is the continuous flow stirred reactor (CSTR) with no biomass recycle, where HRT and solid retention time (SRT) coincide. Other types of reactors, such as packed bed reactors (PBR) and anaerobic sequencing batch reactors (SBR), offer the possibility of decoupling HRT from SRT with possible improvements of the process.

3.4.4 Organic loading rate

The organic loading rate (OLR) represents the mass of substrate fed to the reactor per unit time and volume. Its influence on the production of VFAs from FW has not been extensively studied so far. Jiang et al. (2013) reported an increase in overall VFA concentration with OLR, although with a decrease in VFA yield. These results are in agreement with Lim et al. (2008) who noted that although high concentrations of VFA can be achieved at OLRs > 11 g_{TS} L^{-1} d^{-1}, fermentation broth can become very viscous, making reactor operation difficult and leading to the failure of the process; limiting OLR to less than 11 g_{TS} L^{-1} d^{-1} proved more suitable and VFAs accounted for 96.8% of the soluble COD. Regarding the type of acids produced, Wang and Zhao (2009), although aiming at H_2 production, observed that increasing the OLR from 15.10 to 37.75 kg_{VS} m^{-3} d^{-1} led to reduced acetate and butyrate production and increased propionate and lactate concentrations. Lactate concentration represented 30% of total COD at an OLR of 37.75 kg_{VS} m^{-3} d^{-1} (Wang and Zhao, 2009).

3.4.5 Food to microorganism ratio

The food to microorganism ratio (F/M) affects the metabolic and kinetic characteristics of microorganisms and, therefore, the generation yields of soluble and gaseous products. However, few studies are available on the influence of F/M on acidogenic fermentation of FW and most of them refer mainly to fermentation aimed at H_2 production (Cappai et al., 2014, 2018; Soomro et al., 2019). Cappai et al. (2018) evaluated the effect of F/M on H_2 production and found that a F/M of around 7 g VS_{FW} g $VS_{inoculum}^{-1}$ of led to the maximum H_2 yield, with an associated VFA concentration of 24 g L^{-1}. Due to different overlapping pathways, the fermentation products were observed to include acetate, butyrate, propionate, and ethanol. The highest acetic acid production (2.5 and 3.5 mmol g^{-1} of VS_{FW}) was obtained at F/M of 20 and 4, respectively. The highest butyric acid production (1.5 mmol g^{-1} of VS_{FW}) was observed at F/M = 12.5 and 4. F/Ms of 26.1, 11.1, 4.3 and 0.36 g of $VS_{substrate}$ $gVS_{inoculum}^{-1}$ were applied for the production of VFAs from the organic fraction of municipal solid waste (OFMSW) (FW + paper waste) in a percolation reactor without pH control, by Soomro et al. (2019). The production of VFAs of 14 g L^{-1} (377 mg g_{VS}^{-1}) was found at F/M = 4.3, with a composition dominated primarily by butyric, acetic, and propionic acid. The optimization of F/M helps to predict the yields achievable from a specific substrate and the amount of biomass to be maintained in the system, which is useful in view of process scale-up, in particular for the start-up of fermentation reactors.

3.5 Inhibitors

An important issue to consider for optimizing VFA production is the presence of inhibitors in the FW (such as oil or metal ions), in the inocula, or produced during fermentation (i.e., ammonia, H_2, and soluble products). Liu N. et al. (2017) focused on the negative effects of salt and oil. An inhibition effect occurred at salt concentrations > 6 g L^{-1} and oil concentrations > 5 g L^{-1}, which resulted, respectively, in a 18.7% and 6% decrease in VFA concentration from the control test. In the study by He et al. (2012) on the effect of saline conditions, the highest production of VFA (0.54 g g^{-1} of dry FW weight) was achieved at a NaCl concentration of 10 g L^{-1}; it was approximately 23% lower at 70 g NaCl L^{-1}. As the NaCl concentration increased, the presence of butyric acid decreased from 29% to 3% while propionic acid increased from 6% to 51%, indicating a higher tolerance of *Propionibacteria* to salinity. These results also suggest the possibility to acclimatize microorganisms to salinity.

Although nitrogen is an essential nutrient for biomass growth, high concentrations of free (NH_3) or dissociated (NH_4^+) ammonia have been reported to inhibit fermentation (Bundhoo and Mohee, 2016). Pan et al. (2013) reported an inhibition threshold of 3.5 g N L^{-1} for a F/M of 3.8 and 1.5 g N L^{-1} for a F/M of 8.3 in a study on FW DF aimed at biohydrogen production. Cheah et al. (2019) reported that

NH_4^+–N concentrations ≥ 2.0 g N L^{-1} lead to free ammonia inhibition of acidogenic fermentation of OFMSW under alkaline conditions. Ammonium concentrations over 5 g L^{-1} have been reported to be toxic to anaerobic bacteria (including acidogens) by Lee et al. (2014).

High concentrations of solubilized H_2 could result in fermentation inhibition. Dong et al. (2009) reported that high partial pressures of H_2 can inhibit the conversion of LCFAs to acetate and H_2 or could result in a metabolic shift to lactate, ethanol, acetone and butanol. Another cause of bacteria inhibition involves high VFA concentrations. In their undissociated form, organic acids can permeate through the cell membrane, affecting biomass activity, while dissociated acids increase the ionic strength of the medium, eventually causing cell lysis and resulting in a shift from acidogenesis to solventogenesis as a defence mechanism (Bundhoo and Mohee, 2016). Therefore, the production of reduced solvents such as ethanol and butanol works as a detoxification method of the biomass to avoid inhibition caused by high VFA contents and low pHs in the system (Valdez-Vazquez and Poggi-Varaldo, 2009). Nevertheless, solvent production was observed also at pH levels above 5.7, due to the synthesis or activation of the enzymes required for solvent production (Khanal et al., 2004). The inhibition caused by acid accumulation could be prevented by optimising the OLR and removing VFAs continuously, or at least before the inhibition threshold is reached. To this aim, various techniques have been explored integrating the most suitable separation technology with the fermentation process (Arslan et al., 2017; Dessì et al., 2020). Moreover, continuous VFA removal from the fermentation reactor may enhance the production rate and prevent the consumption in internal conversion reactions, especially when mixed cultures are involved (Atasoy et al., 2018). Arslan et al. (2017) showed that the productivity of VFA increased 1.4 times when fermentation was coupled with the recovery of VFA in situ with electrodialysis.

4. RECOVERABLE NON-VOLATILE CARBOXYLIC ACIDS

In addition to VFAs, other carboxylic acids such as lactate and succinate can be produced via fermentation. However, most of the studies performed so far on acidogenic FW fermentation by MMC have focused on the production of acetic, propionic, butyric, and valeric acids, and only a few targeted other valuable carboxylic acids that, conversely, are often produced using pure microbial cultures, pure substrates, or specific components extracted from residual substrates.

Hafid et al. (2010) obtained a maximum organic acid production of 48.64 g L^{-1} from fermentation of kitchen waste at 37°C and pH 5, with lactic acid as the main fermentation product (37 g L^{-1}, or 76.2% of total VFAs), followed by acetic (17.7%) and butyric acids (6.1%). Kim et al. (2016) studied the effect of temperature on lactic acid production from FW at pH 5 using an indigenous mixed culture. Lactic acid was produced predominantly at 50°C and 1 d HRT, accounting for more than 95% of the total VFA production; a maximum concentration of 40 g L^{-1} was observed, corresponding to a lactic acid yield of 1.6 mol mol_{hexose}^{-1} fed to the reactor. Tang et al. (2016) investigated the effects of pH, temperature, and OLR on lactic acid production from FW, without inoculum addition; the highest concentration of 32.8 g L^{-1} (corresponding to a yield of 0.46 g g_{TS}^{-1}) was achieved at 37°C and pH 6. Thermophilic conditions (55°C) and a high pH of 10 adversely affected the production rate and yield, the latter probably due to the partial conversion of lactic acid to VFAs or CH_4 (Komemoto et al., 2009; Li et al., 2014). The concentration of lactic acid gradually increased with increasing OLR and, according to Tang et al. (2016), the process can be operated steadily at an OLR up to 18 g_{TS} L^{-1} d^{-1}, while another increase can have negative effects on production yield. Wu et al. (2015) reported that acidic conditions (pH = 4) can favour the production of lactic acid from mixed fruit and vegetable waste, but the long-term stability of the process requires further investigation. Similarly, Wang et al. (2014) obtained a remarkable lactate-type fermentation at pH 4.0 (18.5 g L^{-1}) and a fermentation time of 20 d.

The use of mixed FW to produce succinic acid has been scarcely reported. The possibility of converting mixed FW into succinic acid was investigated by Sun et al. (2014) by means of pure microbial strains, through fungal and enzymatic hydrolysis with *Aspergillus awamori* and *A. oryzae*, and subsequent fermentation by anaerobic *Actinobacillus succinogenes* and aerobic recombinant *Escherichia coli*, used separately. The use of FW hydrolysate as the sole substrate in *E. coli* aerobic fermentation led to the production of 29.9 g L^{-1} of succinic acid whilst the overall yield was 0.22 g g_{FW}^{-1}. Dessie et al. (2018) obtained a similar production (27 g L^{-1}) generated by *A. succinogenes* using fruit and vegetable waste hydrolyzed by crude enzyme mixtures. Zhang et al. (2013) used bakery waste for the production of succinic acid in fermentation of *A. succinogenes,* obtaining a yield of 0.35 and 0.28 g $g_{substrate}^{-1}$, respectively. Leung et al. (2012) obtained a higher yield of 1.16 g $g_{glucose}^{-1}$ from waste bread fermentation, hydrolyzed with *A. succinogenes*, corresponding to an overall yield of 0.55 g g_{bread}^{-1}.

5. STATISTICAL ANALYSIS AND CRITICAL INTERPRETATION OF VFA PRODUCTION BASED ON LITERATURE DATA

As shown above, literature studies on VFA production from FW report wide variations for both the overall production yield and the distribution of the different species. To clarify the existing relationships between the process performance and the main variables of concern and identify optimal combinations of these to maximize the VFA yield, available literature results on VFA production from different types of food waste and organic fractions of municipal solid waste were collected and processed statistically. In order to derive a reliable and consistent dataset allowing for mutual comparison of process yields and identification of the optimal region for VFA production, the results retrieved in the references considered in the present paper were screened for thoroughness and consistency of the information provided and converted into homogeneous units of measure in view of further processing. A selection of the variables explored in a consistent number of previous studies to provide a significant statistical sample was performed. To this regard, it should be mentioned that additional parameters may in principle have an influence on the process, but the amount of information that can be retrieved in the existing scientific literature is currently not adequate to allow for reliable predictions. The screening procedure resulted in 295 individual data points from 39 different publications that were used for the statistical analyses. The input variables used in the analysis and their corresponding levels are reported in Table 2 and Figure 2, while the whole set of data gathered from the selected literature references is reported in Table 1SM.

The total VFA concentration and the associated production yield were assumed as the response variables for the analysis. Their statistical distribution is shown in Figure 3a and b. Figure 4 reports similar data for the VFA yield grouped by level of qualitative input variables (reported only for levels with at least 20 data points available). The data in Figures 3a and b indicate maximum values for the VFA production yield and concentration (excluding the outliers of the distribution) as high as 25 $g_{tot\ VFA\text{-}COD}$ L^{-1} and 1.1 $g_{tot\ VFA\text{-}COD}/g_{VS}^{-1}$. The grouping shown in Figure 4 provides further information about the VFA yield for fixed categories of each input variable. Within each category, a comparison of the yields on average terms can be made: more specifically, the highest average process yield was displayed: for the substrate type, by food waste from canteens/cafeterias/restaurants and source-separated OFMSW; for the inoculum type by activated sludge and acclimated mesophilic acidogenic biomass; for the inoculum treatment by thermal pre-treatment; for the temperature regime by mesophilic conditions; and for the pH control method by uncontrolled pH conditions. It should however be noted that the data also display large ranges of variation, with significant deviations from the average value. This reflects the underlying influence of the numerous process parameters described in the previous sections, with potential individual effects as well as mutual interactions of either synergistic or antagonistic nature that cannot be directly identified from Figures 3 and 4. It therefore makes little sense to study the separate effects of each relevant parameter on VFA production, while elucidating their joint influence becomes crucial in order to identify the optimal conditions in view of full-scale implementation.

Table 2 here

Figure 2 here

Figure 3 here

Figure 4 here

In order to provide further insight into the parameter combination that maximizes VFA production, a recursive partitioning approach (Hornik et al., 2006) was used as the data processing methodology to allow for classification of the VFA production results on the basis of the process variables of concern listed in Table 2. The output of the analysis is commonly provided in the form of a binary regression tree that identifies the relevant variables that influence the response (explanatory variables), at the same time singling out the existing associations among these. The regression tree is derived by recursively splitting the original sample into a pair of clusters that have the smallest within-cluster distances in a defined metric. The two generated clusters (son nodes) are then further divided on each branch according to the same grouping criterion. The splitting procedure is stopped when the null hypothesis of independence between any of the input variables and the response can no longer be rejected; the node at which this condition occurs becomes a terminal node of the tree. In other terms, recursive partitioning isolates statistical groups having progressively reduced size and increased internal homogeneity in the values of the selected response variable.

The data points were processed using the *partykit* package implemented in the statistical software R (Hothorn and Zeileis, 2015). The output of the analysis was graphically depicted as a regression tree for which the number of data points of the response variable and their respective statistical distribution (represented through a box plot showing the 25^{th}, 50^{th} and 75^{th} quantiles as well as the average value) are reported at each terminal node. The results are shown in Figures 5a and b for the total VFA concentration and yield, respectively. It should be noted that the conclusions derived from the statistical analysis are only valid within the explored ranges of the investigated variables (see Table 1SM), while nothing can be inferred about the process performance outside such ranges.

As far as the VFA concentration was concerned (see Figure 5a), the hierarchy of grouping of the experimental data was found to be dictated by the pH control method, the inoculum pre-treatment and the substrate type. More specifically, nine terminal nodes were identified, which displayed different average values and statistical distributions. The highest total VFA concentrations (average = 28.2 g_{COD} L^{-1}) were found for node 17 (11 data points), which was obtained by splitting first in the pH control method (continuous pH control, continuous control below pH 5.0 only and uncontrolled pH) at the highest hierarchical level and in the inoculum pre-treatment (no inoculum added, no pre-treatment) at the second hierarchical level. On the other hand, the lowest VFA concentrations were observed in node 3 (24 data points; average = 1.97 g_{COD} L^{-1}), corresponding to the use of a buffer solution as the pH control method, and node 7 (41 data points; average = 3.06 g_{COD} L^{-1}), obtained by splitting on the pH control method (continuous pH control, continuous pH control below 5.0 only and uncontrolled pH), the inoculum pre-treatment conditions (no pre-treatment, thermal pre-treatment), and substrate type (activated sludge, food waste + sludge, OFMSW + sludge, wastewater).

Figure 5 here

The analysis of the total VFA yield (see Figure 5b) identified, in order of decreased ranking, the following variables and related thresholds for maximization of the VFA production: F/M ratio (≤ 1.6 g VS_{FW} g^{-1} of VS_{inoc}), pH control method (no pH control), pH value (> 5.7) and test duration (< 7 d – batch conditions). This optimal combination of conditions, corresponding to node 22 (7 data points), was found to result in the highest average VFA yield (1.2 g_{COD} g_{VS}^{-1}). On the other hand, the lowest VFA yield (0.14 g_{COD} g_{VS}^{-1}) was observed to correspond to node 10 (15 data points), separated according to the following conditions for the input variables: F/M ≤ 6 g VS_{FW} g^{-1} of VS_{inoc}, pH control through buffer addition or continuous/discontinuous control, continuous reactor operation, inoculum consisting of acclimated acidogenic biomass (mesophilic) or primary sludge. It must however be noted that, as shown in Figure 5b, other combinations of the input variables (specifically, those represented by nodes 8, 16, 23 and 26) also displayed low VFA production yields (< 0.3 g_{COD} g_{VS}^{-1}).

6. VFA SEPARATION

The development of efficient techniques for the selective recovery of VFAs from fermentation broth is one of the main technical and economical bottlenecks in biorefineries (Atasoy et al., 2018). Several extraction techniques are available, or under development, for the extraction and fractionation of VFAs. The most applied extraction techniques for organic acids are physical processes (precipitation, adsorption, or liquid/liquid extraction), and membrane/electro-membrane processes (Dessì et al., 2021). Each extraction method entails its advantages and disadvantages, and the choice of the most appropriate recovery process needs to consider the destination of the final product and the purity required for its application. For example, a low-purity VFA outflow might be suitable for applications such as polyhydroxyalkanoates (PHA)-producing processes or as a carbon source for lipid production (Liu J. et al., 2017). In contrast, more sophisticated and expensive separation processes are required to selectively recover marketable VFAs. Among conventional processes, both ionic resins and liquid extractants are mature technologies widely applied for the extraction of organic acids at the industrial scale. They are characterised by high extraction efficiencies and a relatively low cost, although suffering from low selectivity and pH dependence (Reyhanitash et al., 2017). Adsorption yields of VFAs up to 76% were achieved from acidogenic digestate of grape pomace using tertiary amine-based ion exchange resins (Rebecchi et al., 2016). The main disadvantages of resins are related to the energy need for the desorption step, and the rapid exhaustion of the adsorption capacity upon repetitive uses (Reyhanitash et al., 2017). Higher extraction efficiencies (>90%) from fermentation broth can be achieved by liquid-liquid extraction using organophosphates such as tri-n-octylphosphine oxide (Alkaya et al., 2009), although the sustainability of such a process is arguably due to the large use of extractant. Concentration-driven membrane processes such as pertraction and pervaporation have been widely applied for VFA extraction, since they require smaller amount of extractants and have lower operating costs than conventional liquid-liquid extraction. Recently, water has been proposed as a sustainable extractant for concentration-driven recovery of VFAs from fermentation processes through silicone membranes, eliminating the requirement for organic extractants (Dessì et al., 2020; Outram and Zhang, 2018). The process selectivity was dependent on hydrophobicity (i.e., longer-chain organic acids were extracted at a higher rate than shorter-chain acids), but was limited by low efficiency and pH dependency since organic acids crossed the membrane only in the undissociated form. Higher extraction efficiency can be obtained by electrodialysis (ED), a membrane process in which ions are separated by electrical potential differences between cation- and anion- exchange membranes. Jones et al. (2017) employed conventional electrodialysis to recover VFAs from fermentation broths, reporting high efficiencies for removal of VFAs of up to 99% at a voltage of 18 V during a 60 min operation. In-line extraction of VFAs from fermentative reactors by electrodialysis was shown to be beneficial for increasing the H_2 and VFA yields (Hassan et al., 2019; Jones et al., 2017). Hassan et al. (2019) incorporated an ED stack of 20 modules to a FW fermentation reactor through a recirculation loop, increasing the H_2 yield from 65 to 227 mL g_{VS}^{-1}, and the VFA yield from 1.9 to 4.7 g L^{-1}, when applying a potential of 18 V.

7. VFAs AS INTERMEDIATE PRODUCTS

VFAs may also be used as building blocks for further processing, for example, for energy recovery or to produce bioplastics and biolipids. Various applications implemented for the valorisation of VFA mixtures are briefly presented in this Section for completeness' sake.

7.1 Energy recovery

7.1.1 Bioelectricity

When FW fermentation is aimed at the production of bio-H_2, the presence of organic acids in the effluent is considered the mere effect of the partial degradation of the original substrate. This is the origin of the coupling of hydrogenogenic fermentation reactors with bioelectrochemical systems (BES) to convert organic acids into electrical energy or further hydrogen.

The VFA-rich fermented stream could be exploited to produce electricity through redox reactions in a microbial fuel cell (MFC). An MFC is a bioelectrochemical system consisting of two compartments (anodic and cathodic), typically separated by a proton exchange membrane (PEM), and electrically connected through an external circuit. In the anaerobic anodic chamber, exoelectrogenic bacteria catalyze the oxidation of the organic substrate by producing reducing equivalents (electrons and protons) and using the anode as the electron acceptor. Electrons are transferred to the cathode through the external circuit producing electricity, while protons migrate to the aerobic cathodic chamber where they combine with electrons and oxygen to produce water (Figure 6a).

Figure 6 here

Several organic wastes including domestic wastewater (Puig et al., 2011) and industrial wastewater (Sahu, 2019), excess sludge (Jiang et al., 2009) and FW (Jia et al., 2013; Moqsud et al., 2014) have been explored as substrates in MFC. When FW is used directly, substrate hydrolysis is indicated as the rate-limiting step in electricity production (Feng et al., 2016), highlighting the need for proper pre-treatment. Acidogenically fermented waste can be used for electricity generation without any further treatment. Rikame et al. (2012) used a FW fermentation broth with a substrate concentration of 5 g_{COD} L^{-1}, obtaining a maximum power density of approximately 15 W m^{-3} and 1.12 V; moreover, 90% COD removal was achieved. Microbial inhibition was observed in the anodic chamber at a substrate concentration up to 20 g_{COD} L^{-1}. The worsening of performance derived from high OLR values was also observed by Mohanakrishna et al. (2010) who used the outflow from an acidogenic sequential batch biofilm reactor (AcSBBR) fermenting vegetable market waste as substrate for a single chambered MFC. By adopting decreasing values of the OLR (3.13, 1.91, 0.93 kg_{COD} m^{-3} d^{-1}), the best performance (0.31 mV, 362.86 mA m^{-2}, 80% COD removal, 176.35 J kg^{-1} COD removed) was observed for the lowest value and attributed to less interferences (e.g., electrode polarization); interestingly, voltage and power improved by 16% and 68% as compared to the use as substrate of unfermented vegetable waste.

The amount of energy harvested in the MFC depends on the composition of VFAs (Venkata Mohan et al., 2019). Teng et al. (2010) report that higher power densities were attained with acetate as the main component, whilst butyrate was found to exert a negative impact; power density was more affected by the type of VFAs than coulombic efficiency. Acetate and propionate were rapidly degraded, and thus supported higher power generation than longer chain species. This was confirmed by Choi et al. (2011) and Mohanakrishna et al. (2010). Moreover, the simultaneous presence of different VFAs slowed the degradation rate of individual acids, indicating that anodic microbes compete for different substrates (Choi et al., 2011).

7.1.2 Biohydrogen

Hydrogen and carbon dioxide are the gaseous products of the DF of organic substrates. Further H_2 production may be derived from the fermented effluent rich in VFAs through microbial electrolysis cells (MEC) (Liu et al., 2012; Rivera et al., 2015) and photofermentation (PF) (Ghimire et al., 2015; Ghosh et al., 2017; Zong et al., 2009). The MEC configuration (Figure 6b) is completely anaerobic compared to MFC (Figure 6a), and protons released from microbial oxidation of VFAs in the anode are reduced to molecular H_2 in the cathode. As the reaction does not occur spontaneously, an external voltage of at least 0.2 V (theoretically 0.14 V if acetate is used as the anodic substrate) must be applied to overcome the Gibbs free energy barrier (vs. 1.8 V for water electrolysis).

Moreover, removal of eventual ammonium is possible as it is transferred through the cation exchange membrane from the anode to the cathode compartment where it can be recovered as ammonia gas by means of stripping and subsequent absorption. Several studies have been carried out mainly using synthetic chemicals or effluents rich in VFAs generated from conventional fermentation of domestic wastewater (Liu et al., 2012), while few studies have been conducted using effluents fermented with FW (Cardeña et al., 2018; Yun et al., 2018). Liu et al. (2012) used a mixture of VFAs (about 6 g L^{-1}, with 40% acetic acid) from the fermentation of waste activated sludge (WAS), obtaining the highest H_2 yield of 1.2 ml H_2 mg_{COD}^{-1} and an overall H_2 recovery of 120 ml g_{VSS}^{-1} d^{-1}. The results showed that > 90% of acetate and < 90% of propionate were effectively converted to H_2. Rivera et al. (2015) observed a maximum H_2 production rate of 81 ml L^{-1} d^{-1} with an organic removal rate of 85% treating a dark fermentation effluent rich in real VFAs. As assessed in different studies (Lenin Babu et al., 2013; Modestra et al., 2015), the optimal value of potential to be applied for the utilization of VFAs and reduction of H^+ to H_2 falls around 0.6 V. Overall, the MEC has been proven to be resistant and resilient to organic overloads, able to recover steady performance in less than 48 h after the occurrence of stress conditions (Cerrillo et al., 2016).

Purple non-sulphur photosynthetic bacteria (PNSB) can generate H_2 and CO_2 from a wide range of substrates, such as simple sugars, industrial and agricultural waste, under strictly anaerobic heterotrophic conditions and in the presence of light as an energy source for PF (Reungsang et al., 2018). PNSBs show an affinity for VFAs, producing H_2 at higher rates from organic acids than pure sugars (Ghosh et al., 2017) and, therefore, PF has frequently been combined with DF in a two-stage process. The potential of coupling DF and PF for H_2 production from FW was investigated by Ghimire et al. (2015) who observed a 1.75-fold increase in the overall H_2 yield with respect to DF only. Zong et al. (2009) estimated a similar increase by measuring an average H_2 yield of 451 ml g^{-1} of FW and a total H_2 yield of 810 ml g^{-1} by integrating DF and PF.

7.1.3 Biomethane

Anaerobic digestion (AD) aimed at recovering methane-rich biogas is by far the most studied and applied approach for the generation of bioenergy from FW. The process is implemented mostly in a single stage (Dahiya et al., 2018; Oh et al., 2018; Xu et al., 2018). However, the different optimal conditions of the microorganisms responsible for acidogenesis and methanogenesis generally result in suboptimal performance of the single reactor (De Gioannis et al., 2017; Lee et al., 2014). The possibility of operating AD in a two-stage configuration was developed for the purpose of optimizing substrate methanization but has become topical again in recent years due to the interest aroused by the additional possibility of producing bio-H_2 in the first fermentative stage (De Gioannis et al., 2017). FW-derived VFAs are easily suitable for valorisation in the second anaerobic reactor where optimal environmental conditions are established and maintained for slow-growing methanogenic bacteria (pH range 7 - 8, HRT 10 - 15 d). Several authors demonstrated that the two-stage AD configuration may result in 20-25% higher energy recovery than the single-stage one, in light of the improved hydrolysis and fermentation of FW in the first stage, with significant production of VFAs readily available to methanogenesis (De Gioannis et al., 2017; Voelklein et al., 2016). Moreover, the two-stage configuration, allowing enrichment of the methane content by 14 - 17%, could reduce the potential costs for upgrading the biogas to biomethane, as stated by Voelklein et al. (2016) and De Gioannis et al. (2017). The H_2 produced in the first stage may be mixed with methane, forming biohythane, a combustible gas containing 10-15% H_2, 30-40% CO_2, and 50-55% CH_4 that could be further upgraded to biobased hythane by removing CO_2 (O-Thong et al., 2018). The main feature of the two-stage process is the possibility to adjust the inflow to the second stage to control the accumulation of VFAs, which can hinder the methanogenic microorganisms. An inhibitory propionic acid concentration for the methanogenic activity of 1 g L^{-1} was reported by Wang et al. (2009), whilst no significant inhibition effect at acetic and butyric acid concentrations of 2.4 g L^{-1} and 1.8 g L^{-1}, respectively, was observed. Xu et al. (2018) indicate that acetic acid at a concentration between 1.5 g L^{-1} and 2.5 g L^{-1} was the main factor affecting methanogenesis of kitchen waste; the methanogenic activity was completely inhibited at a total VFA concentration of 5.8 - 6.9 g L^{-1}.

7.2 Biopolymers production

Polyhydroxyalkanoates (PHAs) are biodegradable polymers that have received increasing attention in the bioplastic market as a substitute for traditional fossil fuel-based plastics due to their physicochemical properties. Biodegradability, rubbery-like characteristics, better oxygen barrier compared to polypropylene (PP) and polyethylene terephthalate, better water vapor barrier compared to PP, and fat/odour control are well-recognized characteristics. In view of a wide range of applications, including packaging, medical and pharmaceutical applications, energy and fine chemicals (Tsang et al., 2019). PHAs are biologically produced by a wide range of microorganisms as energy storage granules accumulated in their cell cytoplasm under stress conditions caused by the limitation of nutrients, electron donor, or acceptor (Valentino et al., 2018). When the limitation of nutrients is the source of stress, a three-stage process is used to produce PHA from MMC: i) anaerobic-aerobic fermentation (synthesis of VFAs and other organics), ii) selection of MMC and enrichment through an aerobic dynamic feeding system (feast and famine strategy focused on carbon availability), iii) PHA production (Nielsen et al., 2017; Sabapathy et al., 2020). The main factors influencing three-stage PHA production include the structure and metabolism of the microbial community, feeding regimes and type of aeration, culture conditions (pH, temperature, C/N/P ratios, etc.), and substrate characteristics (Sabapathy et al., 2020). As for the substrate, besides the concerns related to the exploitation of refined/food competing feedstock (e.g. sugarcane, vegetable oil), the use of PHAs is still limited mainly by the production cost, 5 – 10 times higher than that of petroleum-derived polymers such as polyethylene (Raza et al., 2018). For these reasons, research efforts are required to move from pure microbial cultures and feedstocks (such as glucose), towards MMC and widely available low-cost feedstock, such as organic waste or activated sludge (Bugnicourt et al., 2014). In this respect, MMC and FW fermentative-VFAs are considered a suitable combination to produce PHAs through the three-stage process, although they are characterized by lower performance compared to pure cultures and selected substrates (Nielsen et al., 2017).

It is also worth mentioning that the most produced PHAs are short-chain poly-3-hydroxybutyrate (PHB) and poly-3 hydroxyvalerate (PHV), and the type of PHAs available depends strictly on the composition of the VFA mixtures in the feed, since the hydroxyvalerate content is known to be proportional to the concentration of propionate and valerate (Amulya et al., 2015). This aspect points at the importance of identifying operating conditions for the fermentative process that allow one to properly address the metabolic pathways, even when the feed consists of complex and heterogeneous substrates such as FW. However, FW has rarely been investigated as the starting substrate for PHA production (Valentino et al., 2018; Wen et al., 2018), compared to the enormous efforts made to convert organic residues of agro-industrial origin, such as waste cooking oil, cheese whey, grape pomace, pea shells, potato peels and olive mill wastewater (Rodriguez-Perez et al., 2018; Tsang et al., 2019). Recent studies performed on PHA production from fermented mixed FW using pure and mixed cultures report a wide range of different accumulation capacities. Hafuka et al. (2011) reported a high PHB content of 87% by continuous feeding of fermented FW to a pure culture (*Cupriavidus necator*), comparable to what obtained by Omar et al. (2011) under fed-batch conditions (84.5%). Eshtaya et al. (2013) observed a lower PHB content (44%) by intermittent feeding fermented FW to pure culture.

Venkateswar Reddy and Venkata Mohan (2012) compared fermented FW from acidogenic H_2 production and raw FW as a feedstock for PHA production; as expected, fermented FW performed better in terms of overall PHA content (39.6% weight/dry cell weight) due to the ready availability of VFAs as precursors; more in detail, a higher content of PHB (61%) was observed, in the form of poly(3-

hydroxybutyrate-co-3-hydroxyvalerate) co-polymer [P3(HB-co-HV)], as compared to PHV (35%). Despite the high VFA conversion (about 90%), lower PHA contents (23.7%) were attained by Amulya et al. (2014) who used VFA-rich effluent from acidogenic FW fermentation as the feedstock to the three-stage process. FW fermentate was used for PHA production also by Wen et al. (2018) whose main interest was understanding the effects of operating parameters such as the organic loading rate (OLR: 1350 vs 8433 mg_{COD} L^{-1}·d^{-1}), and feedstock-related characteristics such as salinity (NaCl: 0, 2.5, 5, 10 and 15 g L^{-1}). Limiting the OLR proved to be necessary to ensure the stability of the process, and although relatively fast kinetics were observed for 5.0 g NaCl L^{-1} at low OLR, a maximum PHA content of 33.4% was achieved at salinity values < 2.5 g NaCl L^{-1}. Valentino et al. (2018) obtained a PHA content in the range 39 – 52%, similar to that obtained by Colombo et al. (2017) (40 - 48%) from acidic OFMSW fermentation using MMC.

7.3 Biolipids for biodiesel production

Biodiesel is usually produced from lipids/oil sources obtained from harvested biomass such as rapeseed, palm, corn and soybean (Gui et al., 2008). However, such substrates raise the ethical concern of using food for fuel, making the identification of alternative lipid sources necessary. Microbial lipid production by oleaginous microorganisms is a promising option. In particular, oleaginous microorganisms belonging to the genera of microalgae, yeast, fungi, and bacteria can directly convert some organic acids into acetyl-CoA, a central intermediate in lipid synthesis, which is then used for the biosynthesis of polyunsaturated fatty acids and microbial lipids in oleaginous yeast cells (Ratledge, 2004). The amount of harvested lipids and their composition vary depending on the strains, culture conditions, and carbon sources (Easterling et al., 2009). So far, most studies on lipid production by oleaginous microorganisms have been carried out using traditional carbon sources such as glucose (Steen et al., 2010), glycerol (Easterling et al., 2009), or pectin and lactose (Papanikolaou et al., 2007). Given the high price of these raw materials, a feasible strategy for cost-effective microbial lipid production is the use of low-cost sources. In this sense, VFAs derived from FW fermentation are envisaged as promising building blocks for lipid biosynthesis to produce oil-based bioproducts (Chi et al., 2011; Gao et al., 2017; Vajpeyi and Chandran, 2015). Chi et al. (2011) used FW dark fermentation effluent aimed at H_2 production as a feedstock for lipid production by *Cryptococcus curvatus* culture, although obtaining a low lipid content of 13.8% (g g^{-1} dry cell weight) due to the high nitrogen concentration in fermented FW effluent. They concluded that high carbohydrate, but nitrogen-deficient, waste streams would serve as better feedstocks for the process. Nevertheless, a similar lipid content (14.9% w w^{-1}) was obtained by Vajpeyi and Chandran (2015) by working with the oleaginous yeast *Cryptococcus albidus* on VFAs produced from FW fermentation. A much higher lipid accumulation of 28.3% w w^{-1} was found using synthetic VFAs under nitrogen-limiting conditions, confirming that lipid biosynthesis is triggered mainly by nitrogen limitation and excess carbon, as also reported by Dahiya et al. (2018). The oleaginous yeast *Yarrowia lipolytica* culture fed with fermented FW yielded an interesting lipid content of 18.2% in the study performed by Gao et al. (2017). Although the feasibility of using FW-derived VFAs for lipid production has been demonstrated, leading to a lipid composition similar to commercial biodiesel feedstock, studies performed using synthetic VFAs showed higher lipid contents (26.1 – 31.6%). The lipid content could also be increased by controlling the feed VFA composition. Fei et al. (2011) investigated microbial lipid accumulation in flask cultures of *Cryptococcus albidus* using synthetic VFAs. The highest lipid content of 27.8% was found by feeding VFA with an acetic/propionic/butyric acid ratio of 8:1:1 compared to ratios of 6:1:3 (27.3%), 7:2:1 (26.1%) and 4:3:3 (19.8%). Gao et al. (2017) studied lipid accumulation in *Yarrowia lipolytica* using synthetic acetic, butyric, and propionic acids, reaching a content of 31.6%, 28.4% and 28.9%, at an initial concentration of 5, 2.5, and 2.5 g L^{-1}, respectively. Higher concentrations of VFAs inhibited cell growth in the following order: butyric acid > propionic acid > acetic acid. Gao et al. (2017) reported that VFAs are not used synchronized but stepwise, since *Y.*

lipolytica first uses acetic acid for lipid production and then uses propionic and butyric acid after its depletion. In light of this, acetate production during fermentation must be optimized to improve lipid yield downstream.

7.4 Biological nutrient removal

Expensive external carbon sources such as methanol, ethanol, or acetate are commonly required to assist the conventional process of biological nutrient removal (BNR) from municipal wastewater through the application of alternate anaerobic–aerobic–anoxic conditions. In order to lower the overall treatment costs, over the last 20 years VFAs have been widely explored as an alternative carbon source for nitrogen and phosphorus removal (Zhang and Chen, 2009). In this context, FW-derived VFAs would be a low-cost option and, being characterized by high C and low N and P contents, the additional unwanted input of nutrients in the process would be negligible, turning to be more suitable for the process than other sources such as primary sludge or industrial effluent (Kim et al., 2017; Lim et al., 2000; Zhang et al., 2016). The required C/N ratio falls within the range of 5-10 mg_{COD} mg^{-1} of N for combined nitrification/denitrification, while 7.5–10 mg of COD are required to remove 1 mg of P (Lee et al., 2014). Lim et al. (2000) used VFAs (mainly acetate) produced from acidogenesis of FW as a carbon source for the removal of N and P from municipal wastewater, obtaining final NO_2^- and NO_3^- concentrations < 1.5 mg N L^{-1}, whilst the concentration of P was reduced to less than 1 mg L^{-1}. Zhang et al. (2016) fed fermented FW obtaining N concentrations < 1 mg L^{-1} in the treated effluent when a COD/N ratio of 6 was applied. A stable denitrification performance of a full-scale wastewater treatment plant with a nitrate removal efficiency of 97.2% was observed by Kim et al. (2017) over a period of seven months during which wastewater from food waste recycling activities was fed as an alternative carbon source; propionate proved to be the most recalcitrant to use, though it was completely consumed after 19 days. Elefsiniotis et al. (2004) stated that the use of acetate allows for a two-fold higher denitrification rate as compared to propionate. In fact, acetate is the first VFA to be consumed and only when its concentration decreases, microorganisms use other VFAs, usually propionate first, followed by butyrate, and finally valerate. The statement is confirmed by Kim et al. (2017) who observed ethanol and acetate being preferred over propionate. This preference for lower-molecular-weight VFAs could be attributed to simpler metabolic pathways (Kim et al., 2017), and would imply, as already stated for biolipid and bioelectricity generation, that to better exploit waste valorization, the DF process should be operated to obtain VFAs of interest for the specific reuse envisaged.

Regarding the removal of phosphorus, it was reported that VFAs obtained from acidogenic fermentation of organic substrates are more effective in the removal of P than synthetic acetic acid (Strazzera et al., 2018). The benefits observed when using a fermentation VFA pool can probably be ascribed to the synergistic effects of other components present in the fermentation effluent (i.e. micronutrients).

8. APPLICATION PERSPECTIVES

The production of acids by fermentation of organic residues has promising implications in view of a full-scale application of DF as the core of waste biorefineries. In this perspective, a dark fermentation-centred layout is proposed in Figure 7, aimed at fostering the recovery of high-value products and energy from FW. According to the simpler implementation option, the DF biogas could be upgraded to ensure sustainable energy recovery in the form of bio-H_2, while the fermentate would undergo liquid/solid separation followed by aerobic biological stabilization of the solid fraction to produce compost. Separation processes would be applied to the liquid fraction of the fermentation outflow to recover marketable VFAs. The purification and separation step of pure organic acids from mixed VFAs is considered to be one of the most relevant aspects in terms of costs and challenges, a major issue between laboratory studies and industrial implementation.

Figure 7 here

More complex configurations could include several downstream VFA processing to produce biolipids, biopolymers, or further H_2 and electricity through bioelectrochemical systems (BES), or bio-methane. Innovative technologies sections as the BES systems hold a great potential and, although have not yet made the leap to the commercial scale and are currently still under evaluation at laboratory scale, could make possible either the selective separation of organic acids using specific anode-cathode separation membranes and combination with electrodialysis cells, or the electrosynthesis of further organic acids by cathodic reduction of carbon dioxide (microbial electrosynthesis).

The theoretic layout, partially based on well-established biochemical processes, is consistent with the need for flexibility typical of the concept of biorefinery as it could be easily and progressively integrated to involve more platforms when other processes should achieve an adequate level of readiness. This is in line with the future needs of a wide spectrum of end bioproducts and could deal in an integrated and flexible manner also with organic waste other than FW, in turn giving the decisive boost to the implementation of waste biorefineries in the framework of a bio-based economy.

However, it cannot be ignored that the full integration of waste management into high-value production systems would require certainty and consistency in terms of feedstock availability and characteristics, process control and, in turn, qualitative and quantitative characteristics of the final products. Furthermore, it would be necessary to take into account that size of the plants, maximum acceptable distances between production sources and treatment plants, need for prompt processing, are very different for waste treatment and traditional biorefineries.

Therefore, the pivotal dilemma, common to every process included in the waste biorefinery concept, is: it is promising, and would be great, but is it actually feasible?

More specifically, is the quality required for products to be commercialized achievable by using FW as feedstock and MMCs?

Considering what has been said on the factors of influence and their mutual interactions, is DF a fully controllable process for the purposes of industrial production if performed using MMCs and a complex and heterogeneous feedstock such as FW? Is it possible to overcome the difficulty of identifying optimal values for the multiple and interconnected influencing factors, even with the help of sophisticated analysis tools such as the statistical ones?

Ultimately, can FW management go beyond the usual goal of environment safeguarding, eventually associated to the recovery of low value products such as compost and biogas, and enter the promising world of bioeconomy?

An interesting indication can come from the analysis of the attempts to scale up the process. As far as the Authors are aware, full-scale plants for the recovery of VFAs from food waste have not yet been built and managed. On the other hand, the analysis of the most recent literature highlights some interesting experiences at the pilot scale; these studies are necessary to bridge science and practice through the assessment of reasonable yields, product quality, and process issues to be expected at the full scale. Valentino et al. (2019) investigated on a pilot scale the dark fermentation of mixtures of OFMSW and sewage sludge (SS) aimed at producing VFAs to be used as substrate for the selection of PHA accumulating biomass and PHA accumulation. The process was performed using a 380 L CSTR operated under thermophilic conditions (42-55 °C) and adopting a HRT of 6 days. The acidogenic performance was considered satisfactory even though some instability in terms of VFAs concentration and distribution was observed at the highest adopted OLR values (about 12.2 g_{TVS} L^{-1} d^{-1}) that affected the system buffer capacity. A better control of pH slightly above 5.0 was attained at lower OLR values (about 6.6 g_{TVS} L^{-1} d^{-1}). The operating temperature did not influence the composition of the VFAs pool, but the thermophilic conditions enhanced substrate hydrolysis; butyric acid was found to be predominant (46% of total VFA), followed by acetic (22%) and propionic (9%) acids.

The same pilot-scale fermenter was used to produce H_2 and VFAs, the latter to be fed to a second anaerobic stage to produce methane (Micolucci et al., 2020).

The reactor was operated for 300 days at 55 °C, adopting a HRT of 3.3 days and an OLR of 19.0 kg_{TVS} m^{-3} d^{-1}, and an original approach to pH control was implemented based on the recirculation of part of the digestate from the methanogenic reactor. Compared to previous studies, reduced pH fluctuations led to higher yields for H_2 and VFAs (about 22 g_{COD} L^{-1}, 33% butyric and 25% acetic acids on a COD basis were the dominant soluble fermentation products).

Yu et al. (2021) performed batch tests on rice-rich food waste using a 120 L pilot CSTR fermenter under thermophilic conditions (50 °C). The HRT and OLR were set at 7 days and 48 g_{VS} $L^{-1}\cdot d^{-1}$, respectively. The results put further emphasis on the role of pH control: the VFAs yield improved by increasing the operating pH from 4.5 to 6.5 (maximum yield: 0.79 mg_{COD} mg_{COD}^{-1}) as result of enhanced substrate hydrolysis. Acetic and butyric acids were the dominant by-products accounting for 55-65% of COD.

Overall, the results of the few pilot-scale studies available show that the production of VFAs from food waste is a feasible process, and that the operating pH is decisive to achieve quantitatively and qualitatively stable process yields. The studies have preferentially used relatively simple reactor configurations such as the CSTR, rather improving the process performance by working in the thermophilic region.

It is also worth emphasizing that the studies conducted on a pilot scale have not aimed at the production of a specific volatile fatty acid, but rather at the recovery of a pool of VFAs to be used directly as a substrate in further biochemical processes such as biopolymers production. This choice seems to acknowledge the difficulties pointed out by laboratory studies in addressing a complex process such as DF towards very specific metabolic pathways, especially when DF is applied to heterogeneous substrates such as food waste. In addition, the recovery of individual VFAs is a very complex task even if it is performed separately and through the innovative combination of physical and chemical processes (e.g., stripping, absorption, adsorption, solvent extraction, nanofiltration, membrane contractor, reverse osmosis and electrodialysis) as reported in Atasoy et al. (2018) and Bhatt et al. (2020). Thus, in order to be technologically feasible and cost effective, the selection of the most suitable recovery strategy at the full scale should consider the final application of the recovered products. In this regard, an application such as biopolymers production, appears to be very promising -at least at the pilot scale- as it does not require complex treatment trains for individual VFAs recovery from the VFAs mixture generated by DF. Thus, further research is needed not only to improve the performance of each “stand-alone” stage of the process but to find out the most appropriate production and recovery stages that -properly integrated- make the overall process of VFAs production and utilization, economically feasible.

9. CONCLUSIONS

The fermentative production of carboxylic acids from organic residues is currently at the laboratory stage and several challenges prevent its full-scale implementation. Some general directions are derived from this review:

- the number of studies specifically dedicated to producing VFAs through FW dark fermentation is relatively low;
- DF requires optimization to achieve high and stable VFA yield, which is critical for downstream applications;
- optimization is not easy given the number of parameters that heavily influence the process and their mutual interactions;
- even the use of optimization tools such as the statistical ones provides controversial answers due to the observed great dispersion of data, which dictated by the variety of conditions that characterizes the available studies;
- the use of MMCs to produce specific VFAs at promising rates needs further investigation;
- the environmental and economic effectiveness of substrate pre-treatment and selective recovery of VFAs must be assessed, and further efforts must be devoted to reducing the overall cost and energy demand to increase the process competitiveness;

- optimisation of downstream biological processes is required to exploit VFAs and provide the desired end product standards;
- pilot-scale studies and a systematic assessment of integrated bioprocesses are required.

Abbreviations

AcSBBR	acidogenic sequential batch biofilm reactor
AD	anaerobic digestion
AK	acetate kinase
ASBR	anaerobic sequencing batch reactor
BES	bioelectrochemical systems
BK	butyrate kinase
BNR	biological nutrient removal
C/N	carbon/nitrogen ratio
CAGR	Compound Annual Growth Rate
CoA	acetyl-coenzyme A
CSTR	continuously stirred tank reactor
DF	dark fermentation
ED	electrodialysis
EU	European Union
F/M	food to microorganism ratio
FW	food waste
HRT	hydraulic retention time
iLDH	NAD-independent LDH
LCFA	long-chain fatty acids
LDH	lactate dehydrogenase
MFC	microbial fuel cell
MMC	mixed microbial cultures
OFMSW	organic fraction of municipal solid waste
OLR	organic loading rate
P3(HB-co-HV)	poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PBR	packed bed reactors
PEM	proton exchange membrane
PF	photofermentation
PHA	polyhydroxyalkanoates
PHB	poly-3-hydroxybutyrate

PHV	poly-3 hydroxyvalerate
PNSB	purple non-sulphur photosynthetic bacteria
PP	polypropylene
PTA	phosphotransacetylase
PTB	phosphotransbutyrylase
SBR	sequencing batch reactors
SCFAs	short-chain fatty acids
SRT	solid retention time
UASB	up-flow anaerobic sludge blanket
VFAs	volatile fatty acids
WAS	waste activated sludge

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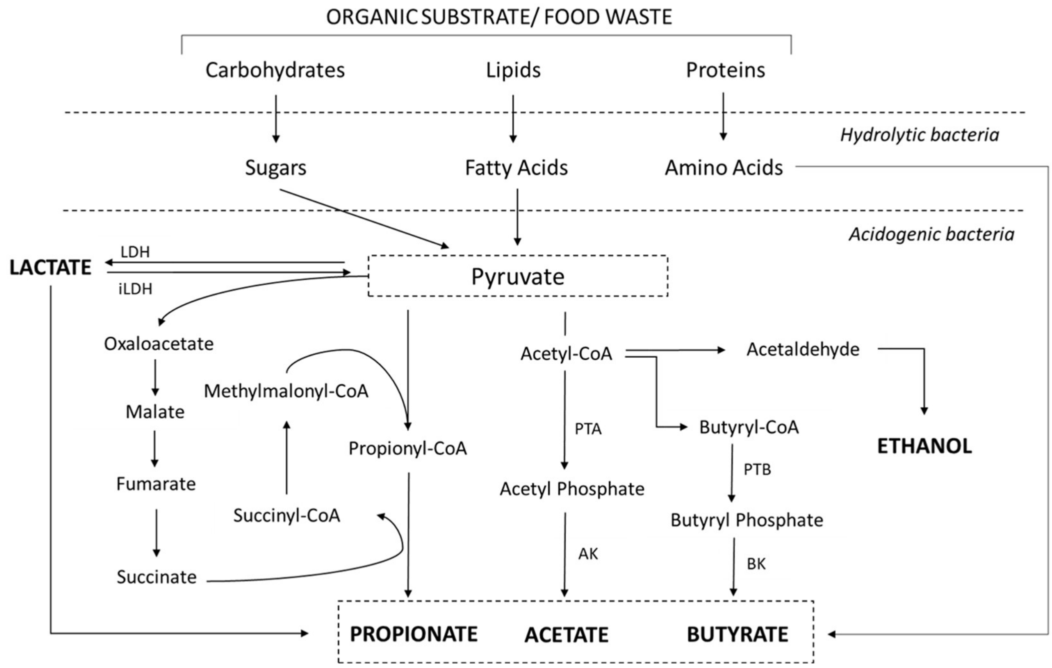

Figure 1. Main biochemical pathways for organic substrate conversion through acidogenic dark fermentation (PTA: phosphotransacetylase; PTB: phosphotransbutyrylase; AK: acetate kinase; BK: butyrate kinase), (adapted from Chen et al. (2013), Dahiya et al. (2018) and Zhou et al. (2018)).

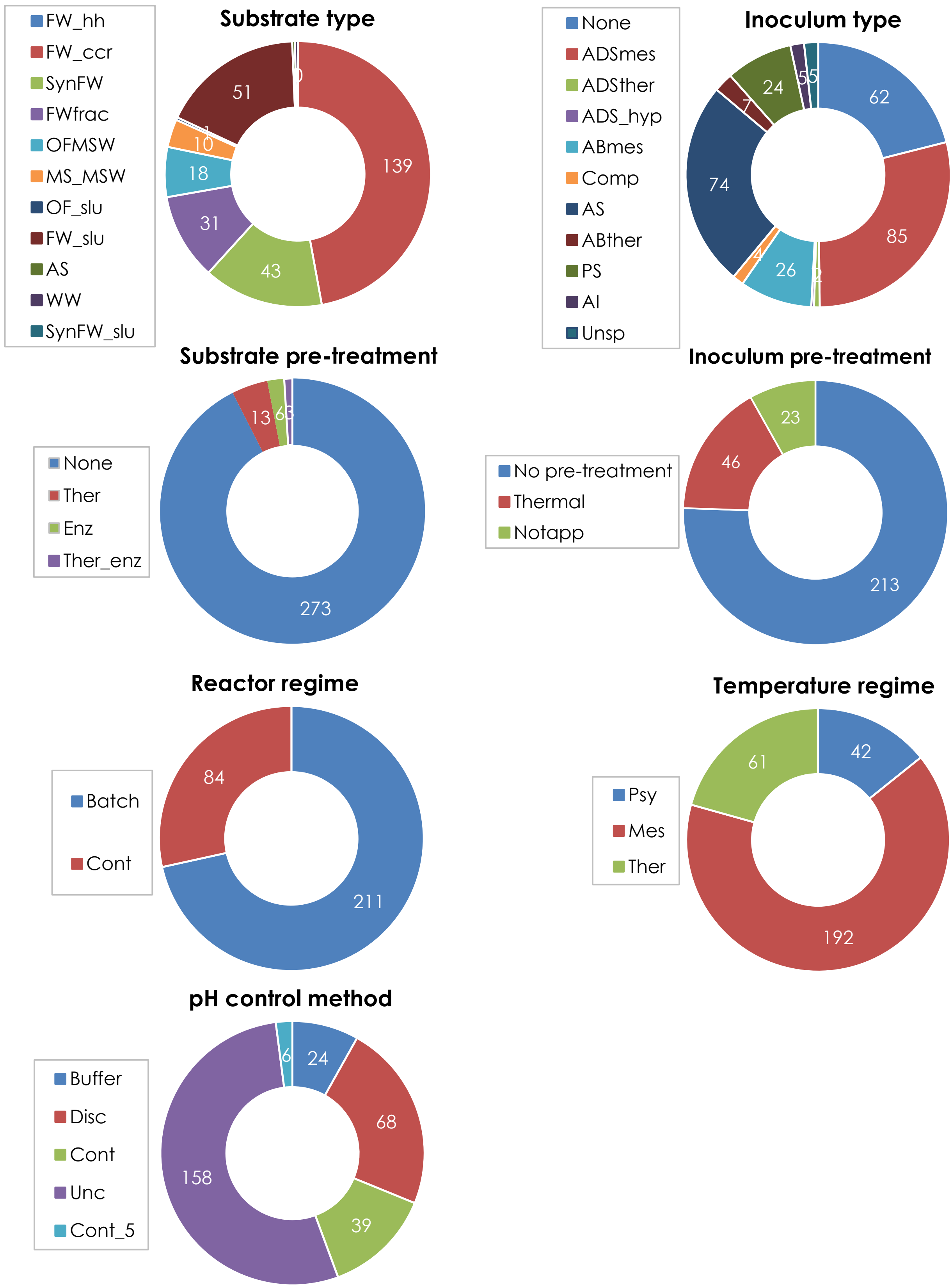

Figure 2. Level distribution of the qualitative variables analysed (labels indicate the number of data points for each level). Note: for abbreviations, please refer to Table 2.

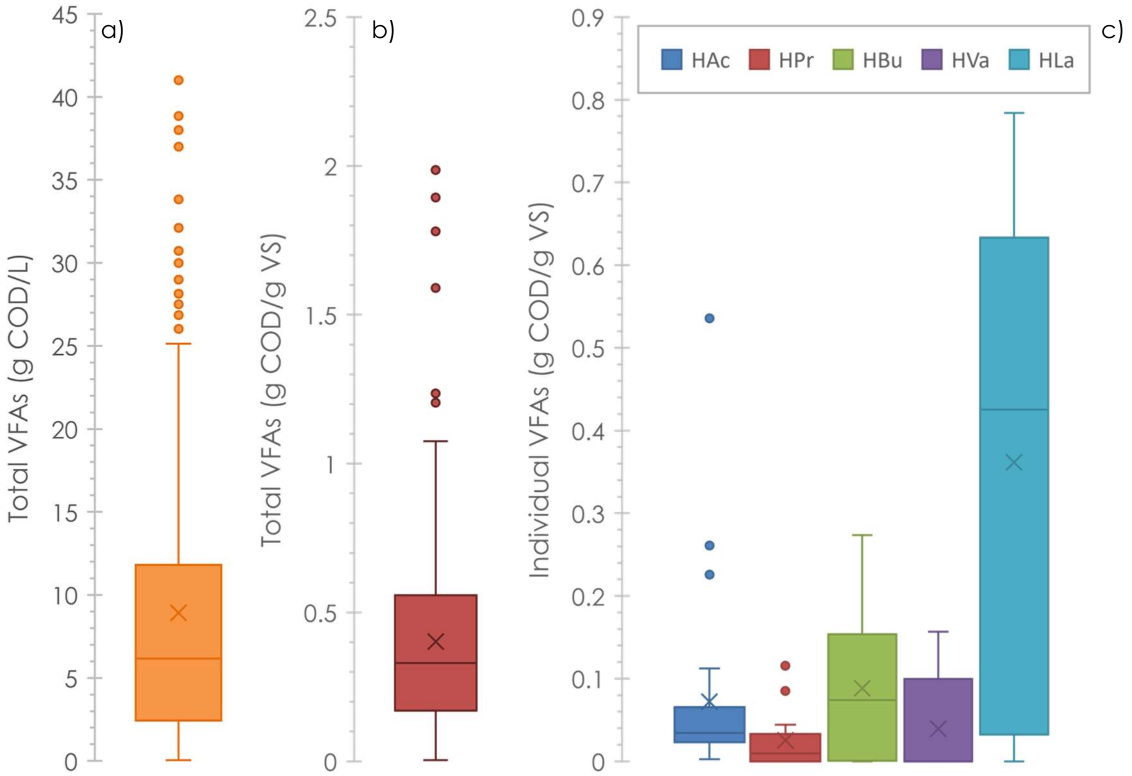

Figure 3. Variation ranges and statistical distribution of the response variables analysed (all input parameters grouped together). Note: boxes indicate the lower and upper quartiles and the median, the whiskers the minimum and maximum data values, × is the average, ○ are outliers.

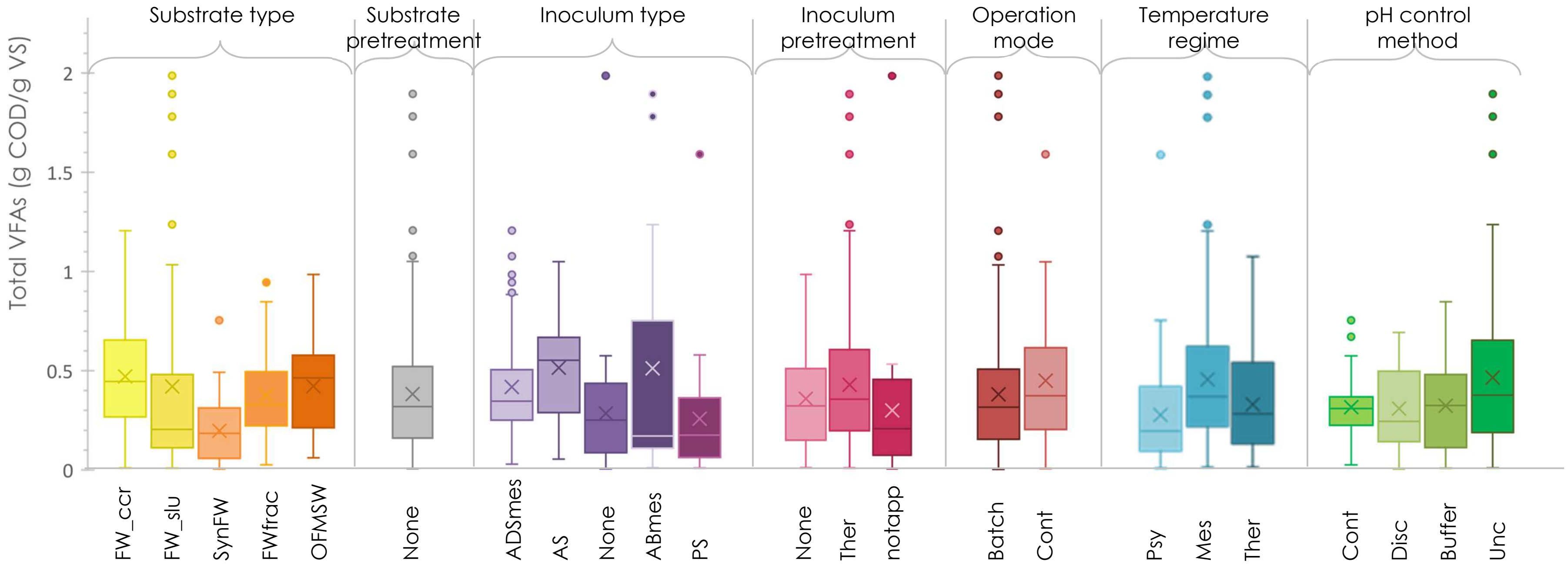

Figure 4. Variation ranges and statistical distribution of the VFA yield grouped by level of qualitative input variables (only levels with >20 data points reported). Notes: boxes indicate the lower and upper quartiles and the median, the whiskers the minimum and maximum data values, × is the average, ○ are outliers For abbreviations, please refer to Table 2.

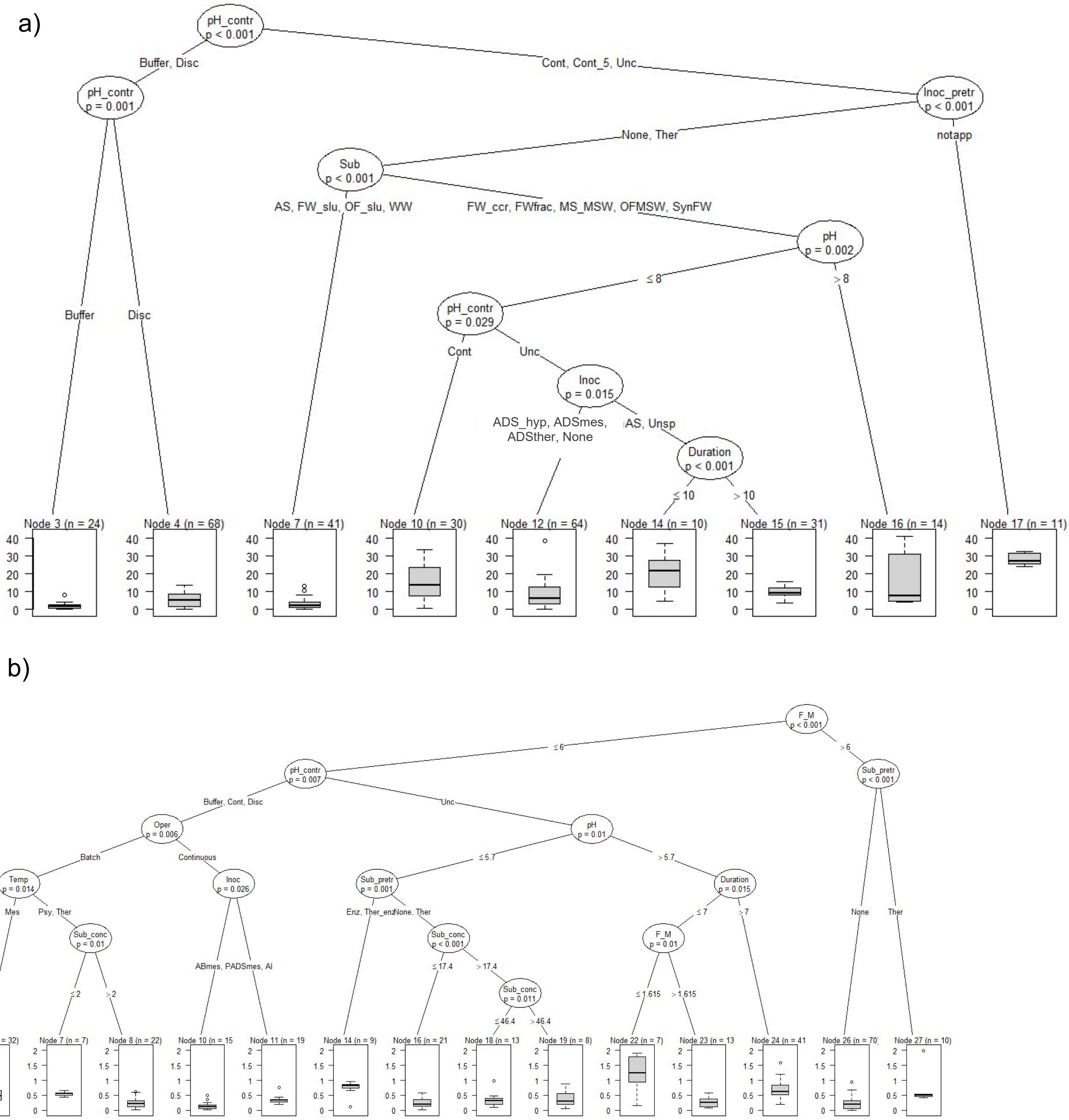

Figure 5. Regression tree identifying the hierarchy of variables effects on: a) total VFA concentration; b) total VFA yield. Note: the variable levels splitting the sub-groups are indicated at each node, while the number of data points of the response variable and their respective statistical distribution are reported at each terminal node. For abbreviations, please refer to Table 2.

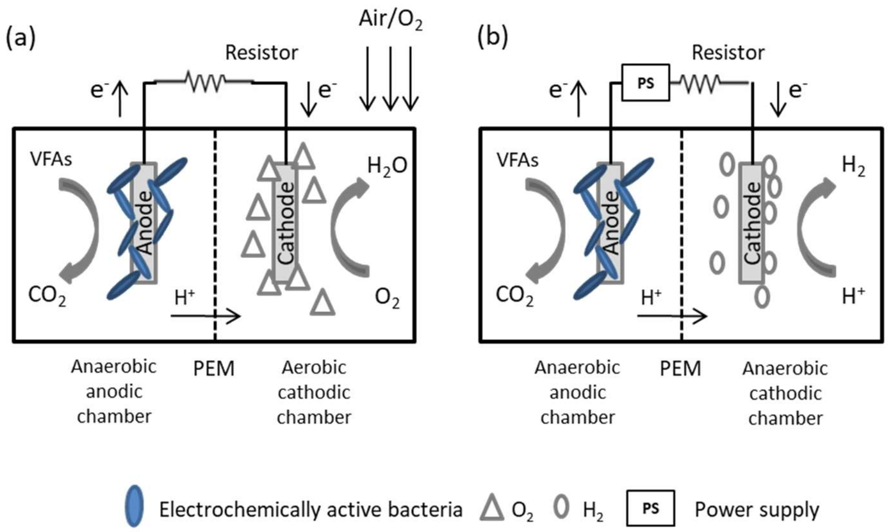

Figure 6. Bioelectrochemical system configuration for: a) electricity generation b) hydrogen production

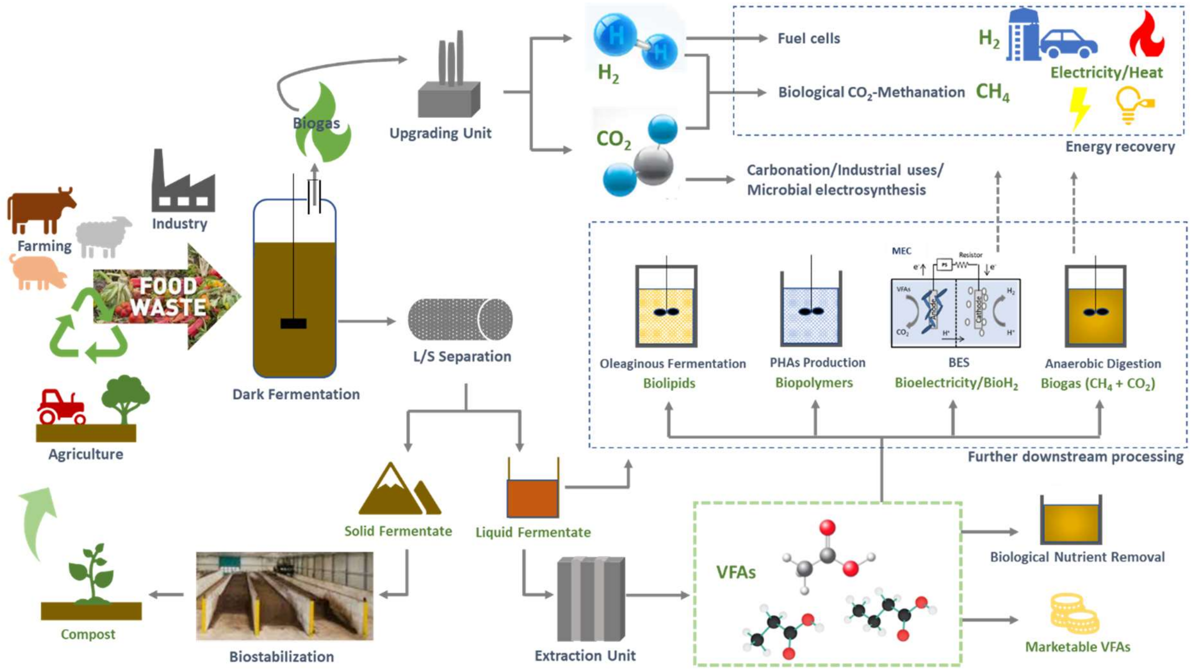

Figure 7. Schematic layout of a fermentation-centered biorefinery approach having VFAs from FW as the main output.

Table 1. Summary of the main metabolic pathways and reactions during dark fermentation

Metabolic pathway and reaction		Eq.
Acetate-type	$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$	(1)
Butyrate-type	$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$	(2)
Propionate-type	$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$	(3)
Mixed-acid	$2C_6H_{12}O_6 \rightarrow CH_3COOH + CH_3CH_2COOH + CH_3CH_2CH_2COOH + 3CO_2 + 3H_2$	(4)
Acetate-ethanol type	$C_6H_{12}O_6 + H_2O \rightarrow CH_3CH_2OH + CH_3COOH + 2H_2 + 2CO_2$	(5)
Homoacetogenesis	$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$	(6)
Lactate-type	$C_6H_{12}O_6 \rightarrow 2CH_3CH(OH)COOH$	(7)
	$C_6H_{12}O_6 \rightarrow CH_3CH(OH)COOH + CO_2 + CH_3CH_2OH$	(8)

Table 2. Input variables used for the statistical analysis of VFA production data

Variable	Symbol	Unit of measure	No. levels [1]	Levels [1]
Substrate type	Sub	---	11	1 = Food waste from household [**FW_hh**] 2 = Food waste from canteen/cafeteria/restaurant [**FW_ccr**] 3 = Synthetic food waste [**Syn_FW**] 4 = Individual food waste fraction [**FW_frac**] 5 = OFMSW (source-separated) [**OFMSW**] 6 = Mechanically sorted MSW [**MS_MSW**] 7 = OFMSW + sludge [**OF_slu**] 8 = Food waste + sludge [**FW_slu**] 9 = Activated sludge [**AS**] 10 = Wastewater [**WW**] 11 = Synthetic food waste + sludge [**SynFW_slu**]
Substrate pre-treatment	Sub_pretr	---	4	1 = No pre-treatment [**None**] 2 = Thermal [**Ther**] 3 = Enzymatic [**Enz**] 4 = Thermal + enzymatic [**Ther_enz**]
Substrate concentration	Sub_conc	$g\ VS\ L^{-1}$	---	---
Inoculum type	Inoc	---	11	1 = No inoculum [**None**] 2 = Anaerobic digestion sludge (mesophilic) [**ADSmes**] 3 = Anaerobic digestion sludge (thermophilic) [**ADSther**] 4 = Anaerobic digestion sludge (hyperthermophilic) [**ADShyp**] 5 = Acclimated acidogenic biomass (mesophilic) [**ABmes**] 6 = Compost [**Comp**] 7 = Activated sludge [**AS**] 8 = Acclimated acidogenic biomass (thermophilic) [**ABther**] 9 = Primary sludge [**PS**] 10 = Anaerobic inoculum [**AI**] 11 = Unspecified [**Unsp**]
Inoculum pre-treatment	Inoc_pretr	---	3	1 = No pre-treatment [**None**] 2 = Thermal [**Ther**] 3 = Not applicable (no inoculum added) [**notapp**]
F/M ratio	F_M	$g\ VS_{FW}\ g\ VS_{inoc}^{-1}$	---	---
Operation mode	Oper	---	2	1 = Batch [**Batch**] 2 = Continuous [**Cont**]
pH	pH	unitless	---	---
pH control method	pH_contr	---	5	1 = Buffer addition [**Buffer**] 2 = Discontinuous control [**Disc**] 3 = Continuous control [**Cont**] 4 = Uncontrolled [**Unc**] 5 = Continuous control (below pH=5 only) [**Cont_5**]
Temperature regime	Temp	---	3	1 = Psychrophilic [**Psy**] 2 = Mesophilic [**Mes**]

				3 = Thermophilic [**Ther**]
Test duration	Duration	d	---	---
HRT	HRT	d	---	---
OLR	OLR	$g\ VS\ L^{-1}{\cdot}d^{-1}$	---	---

[1] Only for qualitative (discrete) variables