Current perspectives on acidogenic fermentation to produce volatile fatty acids from waste

Maria Ramos-Suarez · Yue Zhang · Victoria Outram

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Abstract Volatile fatty acids (VFAs) are key platform chemicals used in a multitude of industries including chemicals, pharmaceuticals, food and agriculture. The current route for VFA production is petrochemical based. VFAs can be biologically produced using organic wastes as substrate, therefore directly contributing to a sustainable economy. This process is commonly known as acidogenic fermentation (AF). This review explores the current research on the development of AF processes optimized for VFA production. Three process steps are considered: feedstock pretreatment, fermentation, and primary product recovery with a focus on in situ recovery. Pretreatment is required for recalcitrant feedstocks, especially lignocellulosic substrates. Different pretreatment techniques for AF application have not been studied in depth. The operational parameters of AF (temperature, pH, hydraulic retention time, substrate concentration, etc.) highly influence microbial activity, VFA yields and product distribution. Optimum conditions are ultimately dependent on substrate composition, however, there is indication that certain operational ranges are beneficial for most feedstocks. VFA recovery and purification are necessary for chemical applications. When recovery is performed in situ, it can help relieve product-induced inhibition and keep alkalinity levels stable enabling further waste degradation. Many techniques have been tested, but none are directly compatible with the fermentation conditions tested. Bio-VFAs have the potential to aid in developing a circular economy, but further development is required. Processes need to be developed with the product market in mind, considering both process integration and systematic process optimization.

Keywords Acidogenic fermentation · Volatile fatty acids · Carboxylic acids · Product recovery · Waste feedstocks · Short chain fatty acids

1 Introduction

Volatile fatty acids (VFAs), or short-chain fatty acids (SCFA), normally refer to C2–C5 fatty acids. Table 1 shows the chemical properties of VFAs with some current market data. VFAs are platform chemicals used in a broad range of industries such as pharmaceutical, food, chemical and agriculture (Baumann...
and Westermann 2016), with over 1.2 billion tonnes produced in 2016 in the US alone. VFAs are predominantly produced through chemical synthesis from petroleum-based feedstocks. This process typically involves high temperatures, high pressures and catalysts for conversion to VFAs (Dionisi and Silva 2016). Oil price instability, finite fossil fuel reserves and the need for a sustainable circular economy, however, are recurring arguments to promote a change from petrochemical production to bio-based processes.

Microbial processes can be used to produce VFAs; either through pure-culture fermentations for a targeted acid or using mixed-culture derived from anaerobic digestion (AD) for mixed VFAs. Microbial VFA production has the advantages of being able to use renewable feedstocks, generating safer products for human health, and offering high product selectivity (in pure culture) (Huang et al. 2007). Mixed-culture fermentation has advantages over pure-culture fermentation as a wider range of feedstocks can be used, including agricultural waste, food waste and wastewater sludges (Lee et al. 2014), as well as offering energy savings through operating under non-sterile conditions. To the authors knowledge, there are currently no commercial mixed-culture AF processes.

AD is a mature technology, and widely used for waste valorization towards biogas (methane and carbon dioxide) production for bioenergy. Low revenues from methane production make it economically unattractive for waste processing companies without subsidies (Smyth et al. 2010; Gebrezgabher et al. 2010; Dolan et al. 2011). Additionally, AD capital expense can be high due to the long retention times and large reactor volume needed to complete metabolic reactions. Consequently, there is a growing research focus on acidogenic fermentation (AF), sometimes known as dark fermentation, to produce VFAs. AF is derived from the first half of the AD process, Fig. 1, and produces a higher value-added product chain. AF can also produce hydrogen and carbon dioxide, further increasing the number of product streams (Kleerebezem et al. 2015).

In this review, the whole process for VFA production from potential market through to the current technology landscape for both the fermentation and primary recovery step is assessed. The current knowledge gaps and challenges which need to be investigated are highlighted, with the aim of progressing a commercially viable bio-VFA platform.

### Table 1 Volatile fatty acid chemical properties and market data

| Acid    | Chemical formula | Molecular mass (g mol\(^{-1}\))\(^a\) | Density (g cm\(^{-3}\)) at 20°C\(^a\) | Boiling point (°C)\(^a\) | pKa\(^a\) | Average price (USD kg\(^{-1}\))\(^b\) | US production in 2016 (MT)\(^c\) |
|---------|------------------|---------------------------------------|---------------------------------------|--------------------------|---------|---------------------------------------|----------------------------------|
| Acetic (HAc) | CH\(_3\)COOH    | 60.052                               | 1.05                                  | 117.9                    | 4.76    | 0.89                                  | 1224–2449                         |
| Propionic (HPr) | CH\(_3\)CH\(_2\)COOH | 74.079                               | 0.99                                  | 141.1                    | 4.88    | 2.20                                  | 11–17                             |
| Isobutyric (iHBu) | (CH\(_3\))\(_2\)CHCOOH | 88.106                              | 0.95                                   | 154.4                    | 4.84    | 2.75                                  | –                                 |
| Butyric (HBu)    | CH\(_3\)CH\(_2\)CH\(_2\)COOH | 88.106                              | 0.96                                   | 163.7                    | 4.82    | 2.55                                  | 1.13–2.27                         |
| Isovaleric (iHVa) | (CH\(_3\))\(_2\)CHCH\(_2\)COOH | 102.133                             | 0.93                                   | 176.5                    | 4.77    | –                                     | –                                 |
| Valeric (HVa)    | CH\(_3\)(CH\(_2\))\(_3\)COOH | 102.133                             | 0.94                                   | 186.1                    | 4.84    | 4.63                                  | 0.45–0.91                         |

\(^a\)Physical properties taken from PubChem Compound Database (Information National Center for Biotechnology 2018)

\(^b\)Price data gathered throughout April 2018 from multiple sources (Alibaba 2018)

\(^c\)Domestic production in the US in 2016 (United States Environmental Protection Agency 2016)

2 VFA uses from acidogenic fermentation

When designing a process, the VFA use needs to be understood, as this will influence the desired process and subsequent optimization. Figure 2 provides
Fig. 1  Anaerobic digestion steps and pathways: encouraged pathways show the preferred metabolic routes for acidogenic fermentation towards volatile fatty acids production

Fig. 2  Examples of potential uses and process options of mixed VFAs from acidogenic fermentation
examples of the possible end products that can be derived from VFAs, and the processing route required to make them from AF. The obvious use for bio-VFAs is as a direct substitute for existing petrochemical-VFAs. Production of bio-VFAs as an alternative to petro-VFAs poses some challenges with the separation of the individual acids from the mixed VFAs in water. This is due to the similar properties of all the VFAs (Table 1), the low concentrations achieved in the fermentation (1–100 g L\(^{-1}\)), and the potential for formation of water-acid azeotropes. An alternative to VFA separation is direct chemical conversion of the mixed VFAs, Fig. 2. Conversion of VFAs to esters has been considered by some groups (Cabrera-Rodrı́guez et al. 2015; Wallis et al. 2017; Plácido and Zhang 2018a). Another possibility could be polymerization of the VFAs for the production of plastics and other fibrous material such as polyvinyl acetate or cellulose acetate butyrate (Straathof 2014). There has been no research into polymerization of mixed VFAs to date.

The primary use of mixed VFAs considered in literature is as a feedstock for biological conversion. As Fig. 2 shows, this covers a wide range of possible uses as a feedstock for the production of polyhydroxyalkanoates (PHA); medium-chain fatty acids (MCFAs); bioenergy and electricity through microbial fuel cells; or for biological nutrient removal (BNR) in wastewater treatment which would produce a fertilizer (Bengtsson et al. 2008b; Mengmeng et al. 2009; Lee et al. 2014; Kleerebezem et al. 2015; Tao et al. 2016; Colombo et al. 2017; Bhatia and Yang 2017; Liu et al. 2018a; De Groof et al. 2019). The VFAs could be converted to biogas, through methanogenesis. This is the equivalent of a two-stage AD process, but with the addition of a separation stage to capture the VFA which is fed to the methanogenesis stage (Lee et al. 2014). Alternatively, VFAs could also transformed into hydrogen as an energy carrier through photofermentation or microbial electrolysis (Uyar et al. 2009). Whilst there is scope for the conversion of VFAs into energy sources, this does not appear to be the most value-adding use of bio-VFAs as there are already proven and well-established technologies for biogas, electricity and hydrogen production from renewable sources.

### 3 Process overview

The production of VFAs can be simplified into three main steps: feedstock pretreatment, fermentation and product recovery, Fig. 3. The chosen waste can be pretreated to enhance hydrolysis and improve the biodegradability. It is then fermented using a mixed-culture to produce VFAs, along with hydrogen, carbon dioxide and soluble by-products, such as alcohols and other organic acids. Ideally, the fermentation will be a continuous process, with maximized yield and productivity. The recovery process can be integrated with the fermentation step to allow maximum carbon conversion to VFAs, therefore the VFA-depleted fermentation broth is recycled to the reactor. Only the primary recovery step is discussed in this review, as any subsequent steps depend on the chosen product use to determine optimal output streams or further purification requirements. The fermentation effluent is likely to contain some soluble compounds in addition to organic suspended solids, therefore still containing chemical oxygen demand (COD) which can be further processed by AD to maximize waste degradation and valorization. The fermentation gasses, CO\(_2\) and H\(_2\), can also be fed to the anaerobic digester for upgrading to methane (Tao et al. 2019).

#### 3.1 Feedstock types

Any organic substrate can be used as feedstock for AF. The preference is for waste biomass (2nd generation) feedstocks avoiding the food vs fuel debate, as well as contributing to waste management strategies (Rulli et al. 2016), therefore this is the focus of AF processes and this review.

The most abundant solid wastes used in AD and AF include animal manure, agricultural residues, livestock residues, sewage sludge, waste activated sludge (WAS), organic fraction of municipal solid waste (OFMSW) and food waste. For liquid wastes; sewage, agroindustrial, chemical industry, food processing and pharmaceutical wastewaters have all been used (Mata-Alvarez et al. 2000; Weiland 2010; Lee et al. 2014; Kleerebezem et al. 2015; Bharathiraja et al. 2018). Recently, novel substrates such as (micro)algae biomass are gaining research interest (Li et al. 2013; Magdalena et al. 2018; Jankowska et al. 2018).

All substrates contain mostly proteins, fats, and carbohydrates. Most compounds in liquid waste are
solubilized organics, but they can also contain suspended solids. Ideally, the feedstock has to be pathogen free and contain a balanced C/N ratio (30/1 to 15/1 recommended for AD) (Gerardi 2003; Paul and Dutta 2018). Animal manures generally have good buffering capacity and contain anaerobic microorganisms suitable for AD, eliminating the need of additional inoculum (Bharathiraja et al. 2018). Most solid wastes comprise a proportion of lignocellulosic material. The lignin structure hinders microbial degradation affecting AD efficiencies (Paul and Dutta 2018). Generally, wastes with high lignocellulosic content require pretreatment to ease the lignocellulose structure breakdown into soluble carbohydrates (Paul and Dutta 2018). This is the case with OFMSW and agricultural wastes. In addition to high lignin content, OFMSW is deficient in nutrients and contains inorganics (Bharathiraja et al. 2018), such as broken glass, grit, plastics and metals. The attractiveness of using algae as biomass for AD/AF reside is the high growth rates compared to terrestrial biomass, CO₂ uptake during growth and removal of nutrients from wastewaters (Bharathiraja et al. 2018). Algae does have drawbacks such as an unbalanced C/N ratio, compounds toxic to microorganisms, and relatively low biodegradability (Matsakas et al. 2017; Bharathiraja et al. 2018).

3.2 Pretreatment

Pretreatment of feedstocks is often required for AF (and AD), as many of the substrates are recalcitrant and do not readily degrade by the micro-organisms alone, e.g. lignocellulosic feedstocks (Romero-Cedillo et al. 2017). Pretreatment studies focusing specifically on VFA production are scarce. Pretreatment studies for AD could be used to infer possible options for AF, although these are not optimized for VFA production.

Pretreatments can be classified as mechanical, chemical, physical, physiochemical, and biological; with the aim to increase biodegradability by fragmenting the lignin, decreasing the cellulose crystallinity or hydrolyzing the carbohydrates into simpler molecules (Jönsson and Martín 2016). Pretreatment should result in acceleration of the hydrolysis stage and VFA yields improvement.

Pretreatment methods for AD have been extensively reviewed by Cesaro and Belgiorno (2014), Ariunbaatar et al. (2014), Romero-Cedillo et al (2017) and Bharathiraja et al. (2018). This review highlights

Fig. 3 Process flow diagram of VFA production via AF. Solid lines show process for maximized yield. Dashed lines show optional AD step to maximize waste valorization, gas upgrading and biomass disposal, if maximal yield is not techno-economically attainable
Table 2: Summary of pretreatment techniques with a focus on application to AF, based on available literature

| Technique                  | VFA/H₂ increases demonstrated | Energy intensive | Most suited feedstock | Additional chemicals required | Accelerates hydrolysis | Toxic compounds introduced or made | Degrades fermentable sugars | Degrades lignin | Recovery/neutralization treatment required | Expensive |
|----------------------------|--------------------------------|-----------------|-----------------------|-------------------------------|-----------------------|-------------------------------------|-----------------------------|----------------|------------------------------------------|-----------|
| Mechanical                 |                                | No              | Dry feedstock         | No                            | No                    | No                                  | No                          | No            | No                                       | No        |
| Acid treatment             | Up to 30% increase in solubility | No              | High protein wastes   | Yes                           | Yes                   | Yes                                 | Yes                         | No            | Yes                                      | Yes       |
| Alkali treatment           | Up to 370% increase in VFA yield | No              | Primary sludge        | Yes                           | –                     | Yes                                 | No                          | No            | Yes                                      | Yes       |
| Peroxidation               | Not tested                      | No              | –                     | Yes                           | Yes                   | Low                                 | No                          | Yes           | No for H₂O₂                               | Yes       |
| Ozoneation                 | Up to 400% increase in VFA yield | Yes             | Lignocellulosic waste | No                            | –                     | Yes                                 | Yes                         | Yes           | No                                       | Yes       |
| Organic solvents           | Not tested                       | Yes             | –                     | Yes                           | –                     | Low                                 | Yes                         | Yes           | Yes                                      | Yes       |
| Thermal treatment          | Up to 380% increase in VFA yield | Yes             | WAS                   | No                            | Yes                   | Yes                                 | No                          | No            | No                                       | Yes       |
| Microwave                  | Small increases on VFA yields (<30%) | Yes             | Sludge                | No                            | –                     | Yes                                 | No                          | –             | –                                        | –         |
| Ultrasound                 | 57% increased solubility         | Yes             | Only tested on WAS    | No                            | –                     | Yes                                 | –                           | No            | –                                        | –         |
| Focused pulsed             | Not tested                       | Yes             | MSW, WAS              | No                            | –                     | –                                   | Yes                         | No            | –                                        | –         |
| Thermochemical             | Up to 470% increase in solubility | Yes             | –                     | Yes                           | Yes                   | No                                  | No                          | Yes           | No                                       | Yes       |
| Wet air oxidation          | Not tested                       | –               | Liquid wastes          | No                            | –                     | –                                   | –                           | –             | Yes                                      | Yes       |
| Ionic liquids              | Not tested                       | Yes             | –                     | Yes                           | –                     | Yes                                 | Yes                         | Yes           | Yes                                      | Yes       |
| Ammonia fiber explosion    | Small increases on VFA yields (<30%) | Yes             | Low lignin content    | Yes                           | –                     | –                                   | Yes                         | –             | Yes                                      | Yes       |
| Biological                 | Fungi                            | No              | –                     | No                            | –                     | Yes                                 | No                          | No            | No                                       | No        |
| Extracellular Enzymes      | Not tested                       | Yes             | –                     | Yes                           | No                    | –                                   | Yes                         | No            | Yes                                      | Yes       |
the pretreatment methods effectiveness for VFA production. The conclusions are summarized in Table 2.

3.2.1 Mechanical

Milling, grinding and chipping increases the surface area, improving biodegradability and solubility. In AD, excessive particle size reduction can accelerate VFA accumulation (Izumi et al. 2010; Cesaro and Belgiorno 2014; Romero-Cedillo et al. 2017). For food waste, smaller particle sizes (0.4 mm) trigger acetic acid production, whereas larger particle sizes (0.9 mm) favor butyric acid (Izumi et al. 2010). Advantages of mechanical treatments are that they require low energy for dry feedstocks, are simple to implement, and improves dewaterability of the waste. The disadvantages are that it does not degrade lignin, does not assist with pathogen removal (Romero-Cedillo et al. 2017) and has high equipment maintenance requirements (Cesaro and Belgiorno 2014).

3.2.2 Chemical

Acid treatment hydrolyses the hemicellulose. This treatment has demonstrated improvements in yield for hydrogen production, which can be correlated to VFA production, and has been proven more effective than other pretreatment options for protein rich wastes (Cesaro and Belgiorno 2014; Romero-Cedillo et al. 2017). For WAS, a 153% and 370% VFA yield increase was observed using hydrochloric acid (Wu et al. 2017) and free nitrous acid (Li et al. 2016), respectively.

Alkali treatment breaks down lignocellulosic structures by dissolving the lignin (Pellera et al. 2016). It, additionally, increases the buffering capacity of the feedstock, preventing pH drops during acidogenesis (Zhang et al. 2014), providing an advantage over acid treatment for AD. There are limited studies on alkaline pretreatment with a focus on VFA production. For lignocellulosic feedstocks, a solubilization rate of 19% was achieved after alkaline pretreatment but this resulted in more than 40% increase in H₂ production (Ozkan et al. 2011). Guo et al. (2011) achieved a 6 times increase in acetic and butyric acid with NaOH pretreatment, although maximum VFA concentrations were low (≤2 g L⁻¹). A study using primary sludge pretreated with different alkali substances found Na₂CO₃ to give a VFA yield 4 times that of untreated sludge. The success was attributed to the increased starting pH (10) which is beneficial for the fermentation and the breakdown of the sludge flocs by the alkali (Lin et al. 2018).

Ozonation uses ozone (O₃) to oxidize, and breakdown, feedstocks and has demonstrated effective delignification of substrates (Travaini et al. 2016; Rosen et al. 2019). Ozone is regarded as safe chemical and environmentally friendly since it quickly decomposes to O₂. The oxidative reactions cause the cell wall of microorganisms to break (oxidative burst), therefore acting as sterilization process (Lone et al. 2019). The disadvantage of ozone treatment is that the high energy demand for ozone production, approximately 12 kWh kg⁻¹ O₃ (Gomes et al. 2019; Rosen et al. 2019). For lignocellulosic wastes, hydrogen production was increased by 158% (Yirong et al. 2015), however it negatively affected the dark fermentation of food wastes due to the degradation of proteins and carbohydrates (Yue et al. 2019).

3.2.3 Physical

Thermal treatment accelerates the hydrolysis step by altering the structure of the insoluble fraction, reducing the viscosity and increasing the sCOD. This shifts the AD process towards acidogenesis and inhibits the methanogens (Ariunbaatar et al. 2015), therefore making it suitable for VFA production. A 680% increase in VFA production has been observed after thermal treatment (100 °C, 60 min) for waste activated sludge when the fermentation pH is kept at 9 (Dong et al. 2016). For neutral pH, the increase is slightly lower (~300%). It should be noted that the authors did not mention the pH of the control experiment (untreated), and therefore, they cannot conclude the increase is due solely to pretreatment, as pH adjustment clearly had a significant effect. Lower VFA yield increases (~55%) were observed for food waste (Yin et al. 2014). Thermal treatment in combination with other treatments such as enzymatic treatment or pre-fermentation treatment could increase VFA yields from food waste by 380% (Kim et al. 2005) and 200% (Yu et al. 2016) respectively.

Microwave irradiation combines thermal and non-thermal effects since the electromagnetic field destructs the crystalline structures and heats the aqueous environment simultaneously. This treatment
is highly energy demanding and, therefore, expensive. For the pretreatment of sludge a 66% H\textsubscript{2} increase was observed (Yang and Wang 2017). Microwave irradiation combined with alkaline addition showed a 30% increase in solubilization and a 400% increase in VFA/H\textsubscript{2} production from lignocellulosic waste (Ozkan et al. 2011).

Several investigations suggest that ultrasound (US) is the most efficient physical pretreatment technique (Dhar et al. 2012; Yeneneh et al. 2013; Deepanraj et al. 2017). The ultrasound waves combine physical and chemical substrate degradation by the collapse of cavitation bubbles and the generation of free radicals. US promotes the production of different enzymes or improves the activities of the existing ones, depending on the case. However, its main limitations are high energy consumption and maintenance cost (Cesaro and Belgiorno 2014). US pretreatment improved digestibility of WAS 28 times in terms of sCOD, consequently enhancing acidification (Zhou et al. 2013). US was also tested on food waste, with a disintegration degree of 57% and a maximum VFA production of 0.98 g COD g\textsuperscript{-1} VS (Wu et al. 2015a).

3.2.4 Physiochemical

Thermochemical treatment involves the simultaneous use of a chemical agent and heat. Kumar and Mohan (2018) observed a 4.7 times improvement in the solubilization of vegetable waste using 1% H\textsubscript{2}SO\textsubscript{4} and autoclaving at 121 °C for 15 min. This resulted in an AF yield of 0.62 g\textsubscript{VFA} per g of reducing sugars (under controlled pH 6). However, a comparison of VFA yield between treated and untreated substrate was not provided. Zhang et al. (2017) used diluted HNO\textsubscript{3} for the pretreatment of lignocellulosic waste (corn stover) and concluded that the pretreatment was successful despite only acidifying less than 10% of the soluble sugars.

Ammonia fiber expansion/explosion (AFEX) consist of the use of ammonia, high temperature (60–100 °C) and high pressure (Brodeur et al. 2011), which provokes decrystallization of cellulose (Romero-Cedillo et al. 2017), hemicellulose hydrolysis and disruption of lignin linkage to carbohydrates (Brodeur et al. 2011). Although studies of AFEX pretreated materials for AD/AF are scarce, it has been demonstrated to improve VFA yields by 21% from lignocellulosic substrates (Blasig et al. 1992).

Ionic liquids (ILs) can be used to dissolve cellulose, which can be recovered by addition of water or ethanol; or extract the lignin, making the substrate more biodegradable (Romero-Cedillo et al. 2017). It typically involves heating in temperature ranges of 80–180 °C, therefore this treatment is classified as physiochemical. Pretreatment with ILs for the production of bioethanol from lignocellulosic substrates has been widely explored (Elgharbawy et al. 2016). To the authors knowledge there are no studies of AF using ILs pretreatment. However, some studies focusing on biogas production indicate ILs can improve AD of lignocellulosic substrates, in some cases (Romero-Cedillo et al. 2017). Pretreatment with 1-ethyl, 1-butyl and 1-hexyl-3-methylimidazolium chloride increased biogas production by 64–140% from different lignocellulosic substrates (Gao et al. 2013). In a study using 1-ethyl-3-methylimidazolium acetate with tomato pomace as substrate, despite the improved enzymatic hydrolysis by cellulase enzymes, pretreated feedstock did not show improved biogas yields. This is presumably due to the formation of inhibitory compounds such as melanoidins and n-derivative amides (Allison et al. 2016). While it can be inferred from AD data that in some cases ILs could improve AF, direct investigation of IL pretreatment for VFA production is recommended.

3.2.5 Biological

Biological pretreatment can be performed with the addition of microorganisms different to AD/AF species, usually fungal, which are better at hydrolyzing the substrate. There have been no studies of fungal pretreatment for AF. Specific extracellular enzymes can also be used, tailored to the feedstock composition. For example, proteases such as trypsin are ideal to hydrolyze protein rich wastes, but this enzyme type can negatively affect acidogenic bacteria, by degrading bacterial proteins (Goldberg 1972; Plácido and Zhang 2018b) and therefore, it is not recommended for VFA production. Consequently, enzymatic pretreatment of variable composition substrates such as OFMSW is often discouraged. Enzymatic pretreatment can be performed in a separate step prior AD/AF or during AD/AF. In the second case, the lifetime of the enzymes can be affected due to the action of endogenous proteases of the AD microorganisms (Odnell et al. 2016). The main advantages of
biological treatments are their non-polluting nature and no waste stream, as the enzymes or biological agent will eventually degrade in AD (Cesaro and Belgiorno 2014; Romero-Cedillo et al. 2017).

3.2.6 Discussion

The discussed pretreatment techniques have demonstrated improved biodegradability of substrates resulting in an increase in VFA or H₂ production, Table 2. Methanogens are more easily affected by toxic compounds than acidogens, therefore if a pretreatment has been discarded for AD it should not be assumed unacceptable for AF.

To enable improvements to the AF process, the pretreatment techniques need to be developed with a specific focus on enhancing VFA production, particularly with novel techniques such as pretreatment with ionic liquids. Alkali/acid treatments are among the most developed chemical pretreatments, with the main disadvantage being toxic compounds generation, however, slight concentrations of toxic compounds might be advantageous to inhibit methanogens. Among the physical techniques, ultrasound looks like the most promising, though it has only been tested on AF of WAS. Biological treatments can be advantageous, as they do not produce toxic compounds like most chemical and physical techniques. Fungi is a cheap viable option to breakdown lignocellulosic feedstock, unfortunately the substrate (organic potential) is also degraded to CO₂ during pretreatment. Extracellular enzymes (microbe-free) have a similar effect but avoiding degradation to unwanted byproducts. However, current commercial enzymes can be expensive. As biological processing and biorefineries develop and become a standard component in the manufacturing landscape, the enzyme market has the potential to expand and offer more affordable options.

Each feedstock presents different challenges in terms of solubilization, thus different pretreatments are recommended. Most of the pretreatment studies of lignocellulosic feedstocks focus on recovering the cellulose and degrading or separating the lignin and hemicellulose. Cellulose is the preferred substrate for bioethanol production, however, AF has the advantage of metabolizing a wider range of substrates including hemicellulose, protein and lipids. Therefore, this aspect should be considered when choosing the pretreatment method. In the case of WAS and microalgae, the challenge resides with the breakdown of the cell walls rather than lignin degradation/removal. Physical pretreatments seem to be the most suitable for this purpose. In the case of food wastes, pretreatment is not a requirement for AD as the long retention time needed for methanogenesis is generally sufficient for the hydrolysis step. However, in the case of VFA production (where shorter HRT are beneficial to inhibit methanogens), pretreatment might be necessary to achieve maximum conversions.

3.3 Fermentation

It is generally accepted by reviewers of AF that most aspects of AF are not well understood (Lee et al. 2014; Kleerebezem et al. 2015; Arslan et al. 2016). These include microbial population of the inoculum; composition of the substrate; as well as fermentation variables (pH, temperature, feeding system, retention times, etc.) and the interactions between them. These factors affect both the product yield and VFAs produced. Majority of experimental studies generate C2–C5 fatty acids therefore this is the focus of this section. The one thing that is agreed upon is that for best AF performance, methanogens need to be inhibited to avoid conversion of VFA to biogas. This can be achieved by addition of inhibitors such as 2-bromoethanosulfophate (Yin et al. 2016) or by operating under unfavorable conditions for methanogens (e.g. pH < 6).

3.3.1 AF operating and performance parameters

Comparison of VFA production studies is difficult due to inconsistencies in the definitions and units of key variables reported by different authors. Important operating parameters in AD, such as organic loading rate (OLR), are not often included in the methodology of AF studies. Different feedstocks have different physical properties and composition, therefore reporting OLR in terms of chemical oxygen demand (COD) or volatile solids (VS) allows for a direct comparison between different feedstock types.

VS percentage is commonly used for solid waste and is ideal for characterizing substrate consumption in the context of AD, whereas in AF a smaller percentage of VS will be converted to CO₂ and it is not an indicator of performance as VFAs also account for VS. COD units are better suited to define VFA
concentrations and yields in order to compare values with other soluble materials present in the fermentation broth such as alcohols and monomers released during hydrolysis. The VFA composition is highly dependent on the substrate, therefore standardizing product concentrations in terms of COD allows for comparison across a range of feedstocks. The ratios of each VFA present should also be provided as this will allow for conversion to concentration and an understanding of product distribution.

‘Degree of acidification’ (DoA) is the amount of soluble COD due to VFA over total soluble COD of the fermented broth (Garcia-Aguirre et al. 2017; Huang et al. 2018). Generally, authors use the term DoA to refer to the VFA yield (Oktem et al. 2006; Alkaya and Demirer 2011; Dahiya et al. 2015; Sarkar and Venkata Mohan 2017). The DoA as defined in this work does not represent the yield of the fermentation, as it does not account for the organic potential of the influent (COD or VS). Instead it is an indicator of the fermentation performance, as it measures the product ‘purity’ within the aqueous solution. Until significant developments in product recovery and purification are achieved, the fermentation step should aim to reach maximum DoA possible. The most accurate yield definition is mass of VFA produced in terms of COD per total COD fed into the system. Additionally, reporting concentrations is essential as it will highly influence the downstream process, however, it is heavily dependent on the OLR in continuous systems or substrate concentration in batch systems.

Table 3 summarizes each study with the most relevant parameters; hydraulic retention time (HRT), temperature, pH, VFA yield and effluent VFA concentration. In this review, all data has been standardized to the same units to allow for analysis and comparison across the literature available. The parameters were calculated using the data provided by the authors and appropriate unit conversion rates (available as supplementary material).

### 3.3.2 Reactor mode

The majority of AF research was carried out using bench scale batch reactors (<5 L). Batch fermentations achieved higher VFA concentrations, but this had a negative impact on the yield potentially due to inhibitory VFA concentrations being reached. Maximum VFA concentration achieved from a batch fermentation is 58 g COD_VFA L^{-1} using kitchen wastes (Zhang et al. 2005) due to a high substrate concentration (125 g VS L^{-1}). However, the VFA yield was not maximized (< 0.5 g COD_VFA g^{-1} VS). Within the same study, a continuous system with solid recirculation (i.e. removal of liquid broth) resulted in a yield increase by 15% compared to batch due to reduced product inhibition (25 g COD_VFA L^{-1}). Other studies of continuous fermentations were, typically, carried out using stirred tank type reactors at bench scale. For different substrates it was observed that a minimum of 4 to 5 times HRT was needed to achieve steady state (Maharaj and Elefsiniotis 2001; Lim et al. 2008; Hong et al. 2009). However, instability was observed after 4 to 5 times HRT in some cases (Bengtsson et al. 2008a; Plácido and Zhang 2018b).

Batch studies are useful to provide information on the capability for AF, but for an industrial process they provide limited information as the implications of OLR, solid/liquid recirculation, wash out of methanogens, inoculum adaptation and product removal. To understand the full potential of the VFA fermentation, progression to continuous fermentations is required. The downside to continuous fermentations is they are not as high throughput as batch fermentations, making it difficult to rapidly optimize and gain understanding of the parameter interactions. This is hindered further by every substrate having a different set of optimum parameters. Limited work has been carried out investigating the transition of an optimized batch AF to continuous AF. One example is Yu and Fang (2001) who studied batch and continuous (upflow reactor) AF of dairy wastewaters at different strengths. From the data provided, it can be observed how the product range is significantly different at low strengths (2 g COD L^{-1}): acetic is the predominant acid in continuous (0.27 g COD L^{-1}), and acetic and propionic in batch (0.2 and 0.25 g COD L^{-1} respectively). However, both reactor types behaved similar at high strengths (20 g COD L^{-1}) with propionic as the predominant acid (0.6 g COD L^{-1} in continuous), followed by acetic (0.45 g COD L^{-1}). This similarity is observed in Fig. 4, when comparing OLR to VFA concentration for the data presented in Table 3. The comparable linearity between batch fermentation (g VS L^{-1}) and continuous (g VS L^{-1} day^{-1}) indicates that the results from batch fermentations could potentially, be used to infer the outcome of a continuous process; although, this needs to be
| Feedstock                  | Reactor type               | HRT (days) | OLR | T (°C) | pH    | Inoculum Type       | S/I Ratio | Other conditions                                                                 |
|---------------------------|---------------------------|------------|-----|--------|-------|---------------------|-----------|----------------------------------------------------------------------------------|
| Waste activated sludge    | Batch 0.5 L               | 7          | 9.9 | 60     | 11    | (controlled)        | Not seeded | N/A 0.02 g Sodium dodecylbenzene sulfonate (SDBS) g^{-1} VS                        |
|                           | Batch 5.0 L               | 9          | 12.9| 55     | 8     | (controlled)        | Not seeded | –                                                                              |
| Primary sludge            | Batch 2.0 L               | 6          | 10.8| 21     | 6.5   | (initial)           | Not seeded | N/A 0.02 g SDBS g^{-1} TS                                                      |
|                           | Batch 2.0 L               | 6          | 9.5 | 21     | 6.9   | (initial)           | Not seeded | N/A 0.1 g Sodium dodecyl sulfate (SDS) g^{-1} TS                                 |
|                           | Batch 3.0 L               | 5          | 11.6| room   | 10    | (controlled)        | Not seeded | –                                                                              |
| Food waste                | semi-CSTR 2.0 L           | 8          | 9   | 35     | 6     | (controlled)        | Digestate | –                                                                              |
|                           | Batch 0.2 L               | 9.3        | 46.1| 37     | 5.5   | (initial)           | Not seeded | N/A Ultrasound + acid pretreatment                                              |
| Leach bed reactor         | 3.5 L                     | 14.0       | –   | 50     | 7     | (controlled)        | Digestate | 96:4 (dry weight) Leachate recirculation                                          |
| Semi-CSTR 4.5 L           | 11.0                      | 7.1        | 35  | 6      | (controlled)       | Digestate | 3:0.8 (v:v) –                                                           |
| Batch 2 L                 | 4.0                       | 9.5        | 35  | 5.5    | (controlled)       | Digestate | –                                                                              |
| Batch 5 L                 | 3.0                       | 110        | –   | 7      | (controlled)       | Digestate | 20 gVS g^{-1} VS                                                           |
| Semi-CSTR 2.5 L           | 3.0                       | 10         | –   | 5.5    | (controlled)       | Digestate | –                                                                              |
| Biofilm reactor           | 20 L (fed-batch)          | 2.3        | 42  | 30     | –     | –                   | Digestate | –                                                                              |
| Batch 0.5 L               | 20.0                      | 60         | 30  | 6      | (controlled)       | Mixed granular sludge and activated sludge | 80:20 (dry weight) – |
| Batch 0.35 L              | 3.0                       | 100        | 35  | 7      | Digestate          | Digestate | 85:15 (wet weight) – |
| Protein rich food wastes  | Semi-CSTR                 | 7.0        | 9   | 40     | unfixed           | WAS       | N/A –                                                                     |
| Kitchen waste             | Batch 0.5 L               | 9.0        | 35  | 30     | 6     | (controlled)       | Granular sludge | 5 gVS g^{-1} VS Tofu, thermal treatment                                         |
|                           | Semi-CSTR + solid         | 3.1        | 15.52| 35     | 7     | (controlled)       | Not seeded | – SRT = 9.3 days                                                             |

1: VFA yield and VFA concentration.
| Feedstock                      | Reactor type | HRT (days) | OLR \(^1\) T (°C) | pH | Inoculum Type                        | S/I Ratio | Other conditions                          |
|-------------------------------|--------------|------------|-------------------|----|--------------------------------------|-----------|-------------------------------------------|
| OFMSW                         | Batch        | 4          | 40                | 14–22 | 4–5 (uncontrolled)                  | Not seeded | N/A –                                     |
|                               | PFR 80 L     | 4          | 42                | 37  | 6.2 (initial)                      | Not seeded | N/A –                                     |
|                               | PFR 80 L     | 6          | 22.4              | 55  | 6.7 (initial)                      | Thermophilic digestate of urban wastes | N/A –      |
| Cattail                       | Batch 2.0 L  | 5          | 4.1               | 40  | 6.9 (controlled)                   | Rumen fluid | 9:1 (v:v) Inoculum: rumen fluid       |
| Palm oil mill effluent        | CSTR 50 L    | 4          | 55                | 30  | 6.5 (controlled)                   | Not seeded | N/A –                                     |
|                               | UASB 12 L    | 0.9        | 11.7              | 35  | 6 (initial)                        | Biomass from acidogenic fermenter of dairy wastewater | N/A –      |
| Olive oil mill effluent       | PBBR 2.5 L   | 1.41       | 18.3              | 25  | 5.5 (initial)                      | Digestate from olive oil mill effluent | N/A culture recycling ratio = 0.97 |
| Wood mill effluent            | CSTR 1.0 L   | 2          | 27.254            | 37  | 5.5 (controlled)                   | Biomass from acidogenic fermenter | N/A –       |
|                               | CSTR 1.6 L   | 1.5        | 2                 | 30  | 5.5 (controlled)                   | Digestate from brewery industry | N/A SRT? |
|                               | CSTR 1.6 L   | 1          | 4.6               | 30  | 5.5 (controlled)                   | Digestate from brewery industry | N/A SRT?  |
| Paper mill effluent           | CSTR 1.0 L   | 2          | 6.5               | 30  | 6 (controlled)                     | Not seeded | N/A –                                     |
| Cheese whey                   | Batch        | 12         | 18.5              | room | 6 (controlled)                     | N/A Ammonia addition (1 g L\(^{-1}\)) |
|                               | CSTR 1.0 L   | 2          | 6.5               | 37  | 5 (controlled)                     | N/A –       |
| Dairy wastewater              | Upflow reactor 2.8 L | 1.0 | 2.8 | 55 | 5.5 (controlled) | Digestate from dairy wastewater | N/A P supplement |
| Gelatin-rich proteinaceous wastewater | Upflow reactor 2.8 L | 0.5 | 2.8 | 55 | 5.5 (controlled) | Digestate from dairy wastewater | N/A –       |
| Slaughterhouse blood          | Semi-CSTR 1.0 L | 7   | 19.0             | 37  | 7.5 (initial)                      | Digestate | N/A –                                     |
| Pharmaceutical wastewater     | CSTR         | 0.2        | 2                 | 35  | 5.5 (controlled)                   | Granular sludge brewery industry | N/A –       |
| Primary sludge + waste activated sludge | Batch 4.0 L | 6 | 10.4 | 21 | 6.7 (initial) | Not seeded | N/A 0.02 g SDBS g\(^{-1}\) TS |
| Primary sludge + starch-rich wastewater | CSTR 3.0 L | 1.25 | 3.6 | 25 | 6.2 (initial) | Not reported | N/A SRT = 10 days |
| Foodwaste + excess sludge     | Semi-CSTR 0.5 L | 8.9 | 8.31 | 7 (controlled) | Digestate | N/A –                                     |
| Foodwaste + waste activated sludge | Batch 5.0 L | 4 | 14.4 | 20 | 8 (controlled) | Not seeded | N/A –                                     |
| Foodwaste + primary sludge    | CSTR 5.0 L   | 5          | 3.2               | room | 5.5–5.9 (uncontrolled)  | NR        | N/A 10% food waste + 90% PS          |
| Sugar industry wastewater + pressed beet pulp | CSTR       | 2          | 3.8               | 35  | 5.7 (uncontrolled)                 | Digestate (concentrated) | N/A 1:1 COD mixing ratio, nutrient addition |
| Feedstock                      | Main acids (% of COD)                        | VFA yield (mg COD\textsubscript{VFA} g VS\textsuperscript{-1}) | VFA yield (g COD\textsubscript{VFA} L\textsuperscript{-1}) | VSS reduction | Ref                  |
|-------------------------------|---------------------------------------------|---------------------------------------------------------------|-----------------------------------------------------------|---------------|----------------------|
| Waste activated sludge        | HAc, HPr (No % reported)                    | 783                                                           | 7.7                                                       | –             | Mengmeng et al. (2009) |
|                               | 45% HAc, 20% HVA                            | 368                                                           | 4.7                                                       | –             | Zhang et al. (2009)   |
|                               | 27% HAc, 23% HPr, 20% iHVa                  | 240                                                           | 2.6                                                       | –             | Jiang et al. (2007b)  |
|                               | 53% HAc, 12% HPr, 17% iHVa                  | 235                                                           | 2.2                                                       | –             | Jiang et al. (2007a)  |
| Primary sludge                | 32% HAc, 26% HPr, 13% iHBu, 16% iHVa         | 85                                                            | 0.9                                                       | 14.60%        | Ji et al. (2010)      |
|                               | 45% HAc, 25% HPr, 13% Hbu, 10% iHVa         | 302                                                           | 3.5                                                       | 38.00%        | Wu et al. (2009)      |
|                               | TVFA reported as HAc                        | 278                                                           | 1.0                                                       | 45.80%        | Maharaj and Elef simitis (2001) |
| Food waste                    | 49% HAc, 24% HPr, 21% Hbu                   | 549                                                           | 39.5                                                      | NR            | Lim et al. (2008)     |
|                               | HAc, Hbu (No % reported)                    | 367                                                           | 16.9                                                      | 20%           | Elbeshbishy et al. (2011) |
|                               | 40% HAc, 60% Hbu                            | 796                                                           | 11.0                                                      | 45%           | Kim et al. (2006)     |
|                               | 17% HAc, 9% HPr, 63% Hbu                    | 330                                                           | 49.0                                                      | 72%           | Hassan et al. (2017)  |
|                               | 24% HAc, 13% HPr, 53% Hbu                   | 496                                                           | 39.0                                                      | NR            | Jiang et al. (2013)   |
|                               | 37% HAc, 16% HPr, 25% Hbu                   | 529                                                           | 5.0                                                       | NR            | Lim et al. (2000)     |
|                               | 46% HAc, 12% HPr, 42% Hbu                   | 265                                                           | 29.2                                                      | NR            | Liu et al. (2017)     |
|                               | 40% HAc, 22% HPr, 37% Hbu                   | 414                                                           | 12.4                                                      | NR            | Pant et al. (2013)    |
|                               | HAc, HPr (No %)                             | 76                                                            | 5.3                                                       | 55%           | Sarkar and Venkata Mohan (2017) |
|                               | 20% HAc, 70% Hbu                            | 855                                                           | 51.3                                                      | 60%           | Wang et al. (2014)    |
|                               | 40% HAc, 40% Hbu                            | 443                                                           | 44.3                                                      | NR            | Liu et al. (2018b)    |
|                               | 35% HAc, 25% HPr, 15% Hbu                   | 867                                                           | 54.6                                                      | NR            | Wu et al. (2015b)     |
| Protein rich food wastes      | 50% HAc, 25% Hbu                            | 613                                                           | 21.5                                                      | 61%           | Shen et al. (2017)    |
| Kitchen waste                 | 22% HAc, 73% Hbu                            | 462                                                           | 57.8                                                      | 82%           | Zhang et al. (2005)   |
|                               | HAc, Hbu (No % reported)                    | 529                                                           | 25.5                                                      | 89%           | Zhang et al. (2005)   |
| OFMSW                         | 80% HAc, HPr (No % reported)                | 35                                                            | 1.4                                                       | NR            | Bolzonella et al. (2005) |
|                               | 76% HAc, 9% HPr, 9% Hbu                     | 128                                                           | 21.4                                                      | NR            | Sans et al. (1995b)   |
|                               | 23% HAc, 34% HBu, 28% HVa                   | 239                                                           | 32.1                                                      | NR            | Sans et al. (1995a)   |
| Cattail                       | 41% HAc, 35% HPr                            | 570                                                           | 2.3                                                       | 75.90%        | Hu et al. (2006)      |
| Palm oil mill effluent        | 41% HAc, 30% HPr, 29% HVa                   | 368                                                           | 20.3                                                      | 63%           | Rahmani et al. (2015) |
|                               | 41% HAc, 59% HPr                            | 490                                                           | 5.2                                                       | NR            | Borja et al. (1996)   |
| Olive oil mill effluent       | 62% HAc, 22% Hbu                            | 414                                                           | 10.7                                                      | NR            | Beccarri et al. (2009) |
|                               | 54% HAc, 37% Hbu                            | 312                                                           | 15.6                                                      | –             | Dionisi et al. (2005) |
| Wood mill effluent            | 51% HAc, 39% HPr                            | 464                                                           | 1.4                                                       | NR            | Ben et al. (2011)     |
|                               | 62% HAc, 32% HPr                            | 437                                                           | 2.0                                                       | NR            | Mato et al. (2010)    |
| Paper mill effluent           | 24% HAc, 48% Hbu                            | 96                                                            | 5.3                                                       | NR            | Bengtsson et al. (2008a) |
|                               | HAc, Hbu (No % reported)                    | 798                                                           | 14.8                                                      | NR            | Jiang et al. (2012)   |
| Feedstock                                      | Main acids (% of COD)                                                                 | VFA yield | VFA                                                                 | VSS reduction | Ref                      |
|-----------------------------------------------|----------------------------------------------------------------------------------------|-----------|----------------------------------------------------------------------|---------------|--------------------------|
| Cheese whey                                   | HAc, Hbu (No % reported)                                                                | 367       | 4.8                                                                 | NR            | Bengtsson et al. (2008a) |
| Dairy wastewater                               | 33% HAc, 22% HPr, 17% Hbu                                                              | 453       | 1.3                                                                 | NR            | Yu and Fang (2000)       |
| Gelatin-rich proteinaceous wastewater          | 14% HAc, 14% HPr, 15% iHbu, 17% HBu, 12% iHVa, 17% HVa                                | 1568      | 2.2                                                                 | NR            | Yu and Fang (2003)       |
| Slaughterhouse blood                          | 21% HAc, 35% Hbu, 27% iHVa                                                             | 967       | 128.6                                                               | NR            | Plácido and Zhang (2018b) |
| Pharmaceutical wastewater                     | 50% HAc, 19% HPr, 32% Hbu                                                              | 794       | 3.6                                                                 | NR            | Oktem et al. (2006)      |
| Primary sludge + waste activated sludge       | 21% HAc, 29% HPr, 14% iHVa                                                            | 174       | 1.8                                                                 | 23.60%        | Ji et al. (2010)         |
| Primary sludge + starch-rich wastewater        | HAc, HBu (No % reported)                                                               | 289       | 1.3                                                                 | 74.60%        | Maharaj and Elefsiniotis (2001) |
| Foodwaste + excess sludge                     | Not reported                                                                           | 393       | 29.1                                                                | NR            | (Hong and Haiyun 2010)   |
| Foodwaste + waste activated sludge            | 32% HAc, 52% HPr                                                                      | 576       | 8.3                                                                 | 61.00%        | (Feng et al. 2011)       |
| Foodwaste + primary sludge                    | 48% HPr, 52% HBu                                                                      | 148       | 2.4                                                                 | NR            | (Min et al. 2005)        |
| Sugar industry wastewater + pressed beet pulp  | 40% HAc, 51% HPr                                                                       | 511       | 3.9                                                                 | NR            | (Alkaya and Demirer 2011) |

1\(^{1}\)OLR (batch: g L\(^{-1}\); continuous: g VS L\(^{-1}\) day\(^{-1}\))

NR: Not reported
experimentally validated. In contrast, fed-batch fermentations do not follow the same response. The higher VFA concentrations in fed-batch AF is expected due to the additional available substrate, compared to batch or continuous (where some unfermented substrate will be removed in the outlet stream). Interestingly, these results have not plateaued out in terms of VFA concentration, meaning that the expected VFA toxicity at high concentrations has not been confirmed by this data. This indicates that the fermentation system can be pushed towards higher VFA concentrations, which will improve the down-stream processing. To aid the rate of development, it would be beneficial to understand how the transition from batch to continuous processing impacts the fermentation parameters.

3.3.3 Inoculum and microbial community

In AF processes, VFA yield and distribution, for a given substrate, are in essence a result of the microbial community and its activity. Microbial community studies can help understand the acidogenic fermentation process and the effect of fermentation conditions. A wide range of microbes contribute to the fermentation, and further research is necessary to associate each microbe type to their specific or main role within AF. Taxonomic identification of communities is typically carried out by 16S rDNA gene sequencing analysis with results shown in terms of relative abundance (Atasoy et al. 2019).

Acidogenic species can be introduced by an inoculum/seed and the substrate or the substrate alone, Table 3. Most AF studies use AD digestate/sludge as the inoculum due to the presence of more acidogens compared to other inoculums such as aerobic activated sludge (Wang et al. 2014; Yin et al. 2016). However, AD sludge contains methanogens which degrade the VFA. In batch fermentations, optimizing the substrate to inoculum ratio (S/I in g VS g\(^{-1}\) VS) can help inhibit methanogens (Guo et al. 2014). A S/I higher than 3–4 is found to inhibit methanogens successfully (Alzate et al. 2012; Guo et al. 2014). Inoculum acclimation can be another strategy to improve batch AF. Plácido and Zhang (2018b) observed a 43% increase in VFA when inoculum was acclimated by operating a continuous reactor for at least three times HRT. Some authors suggest subjecting the inoculum to a thermal treatment to inhibit methanogens. However, there is little information on optimal conditions to carry out this heat shock treatment (Wainaina et al. 2019) and the effect it has on AF. Often, the conditions used seem arbitrary or based on H\(_2\) production (Tampio et al. 2018; Jayakrishnan et al. 2019). 2-bromoethanesulfonate (BES) is commonly used to inhibit methanogens, but its effect on acidogens is not well understood. Yin et al. (2016) found that addition of BES was effective in inhibiting methanogens and had no significant effect on VFA distribution, however, it affected the relative abundance of microbes.

In acidogenic fermentation, microbes (generally bacteria) carry out biological conversion of
macromolecules e.g. protein, carbohydrate and lipids, to VFA and by-products via their metabolic activities. Anaerobic sludge derived from wastewater treatment mostly contains phyla belonging to *Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Chloroflexi* and *Euryarchaeota* (Li et al. 2019; Greses et al. 2020). *Euryarchaeota* phylum encompass methanogens. High abundance of *Firmicutes* is associated with high acidification yields due to their hydrolytic and acidogenic capabilities (Yin et al. 2016; Atasoy et al. 2019; Greses et al. 2020), particularly *Clostridium* genus (Atasoy et al. 2019). *Firmicutes* proliferate when substrate has high carbohydrate content (Iglesias-Iglesias et al. 2019; Greses et al. 2020). The genus with highest number of genes related to amino acid and carbohydrates metabolism are *Pseudomonas* (*Proteobacteria*) and *Clostridium* respectively (Iglesias-Iglesias et al. 2019).

AD sludge with similar microbial composition can be in slurry or granular form, and these physical properties can influence AF performance. AF using large granular sludge (~3.5 mm) resulted in higher DoA and yields from glucose at pH 10 compared to typical slurry form (Atasoy et al. 2019). Large granules were also more effective in AF of cheese industry wastewater (Atasoy et al. 2020). Despite the differences in microbial relative abundance after 5 days of fermentation, the type of sludge did not significantly affect the VFA distribution, suggesting that pH and fermentation conditions have a larger influence over final product distribution (Atasoy et al. 2019). Differences in microbial relative abundance were observed with the same inoculum when either milk or cheese industry wastewater were used, indicating that relative abundance also depends on substrate type (Atasoy et al. 2020). The same observations have been made for AD and biogas processes (Kushchevych et al. 2019), indicating that a period of adaptation for the microorganisms to adjust to the feedstock is likely to be required before stable operation is achieved.

The inoculum will determine the type of microbes introduced in the system, but the relative abundance can be modified by fermentation conditions and substrate type. Just like VFA distribution, fermentation pH and temperature affect microbial relative abundance. In AF of food waste under mesophilic conditions, neutral pH and pH 5 led to high abundance of classes *Clostridia* (>50%) and *Bacilli* (>50%) respectively. At neutral pH, thermophilic conditions increased the relative abundance of *Bacteroidia* compared to mesophilic conditions. At pH 5 the change in relative abundance due to temperature was less significant (Zhang et al. 2020). At mesophilic conditions, pH changes also had significant effect on microbial relative abundance of potato waste fermentation (Li et al. 2019); at pH 6 the major phyla were *Proteobacteria* and *Firmicutes*, whereas at pH 7 and 8 (highest VFA yields), *Bacteroidetes* and *Firmicutes* dominated. The highest *Euryarchaeota* (methanogens) relative abundance was observed at pH 7 (Li et al. 2019). When using sewage sludge as substrate: pH 7–8 resulted in high levels of *Euryarchaeota* (60%) and *Firmicutes* (20%), followed by *Actinobacteria*. These results suggest that, if operating at neutral pH methanogenic inhibition strategies are required for optimum AF (e.g. BES addition or inoculum pretreatment). At pH 9 (highest VFA yield) dominating phyla were *Firmicutes* (60%, mostly *Clostridia*) and *Actinobacteria* (24%). pH 10 led to 35% *Firmicutes*, 39% *Actinobacteria* and 16% *Proteobacteria* (Chen et al. 2017).

It has also been found that different substrates influence the relative abundance, especially at a genus level. Majority of phyla found in AF of food waste (Yin et al. 2016; Zhang et al. 2020) and sewage sludge (Iglesias-Iglesias et al. 2019) belongs to phyla *Firmicutes, Proteobacteria* and *Bacteroidetes*. Under limited aeration (non-strict anaerobic), the two major classes in food waste AF at pH 6 is *Clostridia* (Yin et al. 2016; Zhang et al. 2020) and *Bacilli* at pH 5 (Zhang et al. 2020). Under strict anaerobic conditions classes *Bacteroidia* (phylum *Bacteroidetes*) followed by *Gammaproteobacteria* (phylum *Proteobacteria*) tend to dominate the community (Yin et al. 2016). The presence of BES negatively affects *Proteobacteria*, reducing the relative abundance to insignificant levels (Yin et al. 2016). Greses et al. (2020) investigated cucumber, tomato and lettuce as substrates, all three substrates led to high levels of class *Clostridiales*, but *Ruminicoccus* were only significant in cucumber and tomato fermentations. AF of lettuce resulted in significant levels of *Acidaminococcus*. In contrast with food waste, for AF of sewage sludge (non-strict anaerobic), the most abundant phyla were, *Proteobacteria* followed by *Bacteroidetes* and *Firmicutes* (Iglesias-Iglesias et al. 2019). *Proteobacteria*
is mostly represented by *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacterial* (Iglesias-Iglesias et al. 2019). Apart from pH, temperature and substrate composition, strong correlations between the microbial consortia with organic loading rates, and oxidation–reduction potential, were found (Xin et al. 2018).

Genomic studies allow the quantification of microbial diversity. Microbial diversity, particularly for complex substrates such as food waste, is strongly linked to high VFA yields (Zhang et al. 2020) and performance stability (Xin et al. 2018). Introducing extra substrates (co-fermentation) can improve microbial diversity and consequently improve VFA yields. Xin et al. (2018) demonstrated that by introducing pretreated corn stalk and pig manure as co-substrates to AF of pretreated WAS, microbial diversity improved and VFA yield of AF of the three substrates was almost two fold that of pretreated WAS alone (based on initial soluble COD and maximum VFA concentration (in COD units)).

While mixed cultures are beneficial to metabolize complex substrates, it is likely to result in multiple product solution with fewer applications. Recent research has focused on how to optimize the inoculum and culture to target a single product through bioaugmentation techniques. One approach is to mix different mixed culture inocula to optimize the process for a targeted product and substrate (Ai et al. 2013). A more rigorous approach was taken by Atasoy and Cetecioglu (2020) who studied the bioaugmentation of mixed culture with the introduction of *Clostridium butyricum* monoculture to target butyric acid production from cheese industry wastewater in a sequencing batch reactor. This is achieved by injecting a small percentage of the reactor volume with the aseptically grown target culture. The bioaugmented reactor saw a 4.6 and 2.3 times increase in HBu and total VFA average yield, respectively, compared to the control reactor, resulting in an increase of HBu selectivity from 21 to 61% (Atasoy and Cetecioglu 2020). Results also show improvement with respect to the monoculture fermentation, indicating syntrophic relationships between the mixed culture and *Clostridium butyricum*. Bioaugmentation also helped with overall production stability (Atasoy and Cetecioglu 2020).

### 3.3.4 pH

In AF, pH is a key parameter to control VFA yields and product distribution, controlled by acid/base addition. Optimum pH for methanogens is 7.0. Acidogen bacteria can handle wider pH ranges, therefore pH has a relevant role in AF to minimize VFA degradation. A slightly acidic pH (5–6) improves hydrolysis due to higher hydrolytic bacteria activity (Jiang et al. 2013), and inhibits methanogens, but pH lower than 5 is inhibitory for acidogens (Bengtsson et al. 2008a; Wang et al. 2014). This is due to the high toxicity of VFA in their free acid form, which is more dominant when the pH is less than the pKa (Table 1) (Arslan et al. 2016). The toxic effect could be overcome with continuous in situ product recovery. Alkaline pH (8–10) can also be beneficial (Feng et al. 2011) as it improves substrate digestibility by dissolving lignin (abiotic effect), inhibits methanogens and it offers buffering capacity (Zhang et al. 2009). At pH 11 or higher, the metabolic activity of acidogens slow down due to the toxic effects of strong alkaline conditions (Mengmeng et al. 2009). Alkaline addition can substantially add to operational costs. In contrast, acidic pH can be achieved by accumulation of VFA, depending on the buffering capacity of the fermentation broth. As a general rule, alkaline pH will favor acetic acid production, while acidic pH will favor propionic and butyric acids (Zhang et al. 2009; Wu et al. 2009; Garcia-Aguirre et al. 2017). In some cases, neutral pH (7) showed better VFA yields. For example, gelatin degradation efficiency was 98% at neutral pH compared to 85% at pH 5, resulting in higher VFA concentrations (Yu and Fang 2003). In the case of kitchen wastes, pH 7 also had the highest solubilization rate of 82% in terms of COD, which coincides with highest VFA concentration, compared to less than 70% for acidic or alkaline pH (Zhang et al. 2005). This could be explained by the differences in substrate composition, as hydrolysis of proteins and lipids is optimum at neutral pH (Arslan et al. 2016). These results indicate that the pH has a greater impact on the hydrolysis step, which is widely acknowledged as the second rate limiting step in AD. This further supports that the optimum pH is going to be substrate dependent.
3.3.5 Temperature

Temperature has a significant effect on yield and the type of VFA produced. Typically, fermentations can be classified by operating temperature, as either psychrophilic (≤25 °C), mesophilic (25–45 °C) or thermophilic (>45 °C). Generally, under thermophilic conditions VFA accumulation is higher compared to mesophilic conditions (Yu and Fang 2003; Zhang et al. 2009; Garcia-Aguirre et al. 2017). Thermophilic temperatures can improve the hydrolysis of solid wastes improving overall digestibility, but pH has a greater influence over this compared to temperature (Garcia-Aguirre et al. 2017). Kinetics under thermophilic conditions, however, are slow therefore longer retention times are required to reach maximum product concentrations (Zhang et al. 2009; Garcia-Aguirre et al. 2017). It should be considered that the higher temperature leads to higher operating costs. Higher VFA yields are achieved at mesophilic temperatures, Fig. 5. This indicates there is a better conversion for substrate to product, although the lower VFA concentrations will be less favorable for product recovery. VFA production at psychrophilic temperatures (~10 °C) is feasible but not competitive with mesophilic production (Maharaj and Elefsiniotis 2001), Fig. 5, with lower VFA concentrations and yields achieved.

Temperature has the potential to influence type of VFA produced, but the current results are inconsistent (Zhang et al. 2009); this is probably due to the lack of knowledge on parameters interactions. For example, temperature can have an effect on ammonia release (Jiang et al. 2013) making it difficult to study the independent effect of these variables. Garcia-Aguirre et al. (2017) found that temperature had no significant effect on product distribution for slaughterhouse wastewater and paper mill wastewater, with the same observation was made for protein rich wastewater (Yu and Fang 2003). However, for OFMSW and winery wastewater, butyric acid was predominant (>70% of COD) at acidic pH (5.5) and thermophilic temperature, compared to propionic and acetic predominance under any other conditions (Garcia-Aguirre et al. 2017). This is further supported by Jiang et al. (2013) who also found that thermophilic temperatures promote butyric acid production from food waste at pH 6 (Jiang et al. 2013).

3.3.6 Organic loading rate and substrate concentration

The organic loading rate (OLR) is the amount of organic material fed (in VS) per volumetric unit of the reactor per unit of time. The recommended values for AD range from 2 to 7 g VS L−1 day−1 (Hobson and Wheatley 1994; Gerardi 2003). Using higher values of OLR can help stop methane production and promote acidogenesis. Increasing OLR above the AD threshold (7 g VS L−1 day−1) results in higher VFA concentrations but lower yields (Lim et al. 2000; Jiang et al. 2013; Liu et al. 2018b). Ideally, a compromise between the yield and the VFA concentration should be found. Dry substrates (TS ~ 20%) are preferred to slow down methanogen kinetics and maximize VFA production, although VS destruction is lower (Liotta et al. 2014).
3.3.7 **Hydraulic retention time**

HRT plays an important role in AF. Short HRTs (<10 days) are preferred to wash out the slower growing methanogenic microorganisms (Hobson and Wheatley 1994; Gerardi 2003). However, if the substrate is a solid waste, short retention times will result in lower yields as hydrolysis is, generally, the rate limiting step (Fdez.-Guelfo et al. 2011). Pant et al. (2013) found that to achieve maximum conversion in the fermentation step at least 3 days HRT is required. In batch mode, maximum VFA concentrations are typically achieved in 4–9 days, indicating relatively short retention times are needed.

3.3.8 **Feedstock**

The type of feedstock will affect the output of the fermentation. Comparison on feedstock compositions is difficult due to differences in AF parameters used. Feedstock compositions for the same type can also vary depending on location or season, for example, resulting in high variability in the reported data. There are numerous studies available to compare AD performance for different feedstock types (Chynoweth et al. 1993). Similar studies on AF towards VFA production are scarce. Although AD studies can serve as reference, optimum substrate compositions might be different for AF. Cheese whey, molasses and OFMSW showed higher VFA potentials over wastes such as glycerol, slurry, winery wastewater, olive mill effluent and landfill leachate (Silva et al. 2013). This was further supported by Garcia-Aguirre et al. (2017) who indicate that OFMSW has the highest VFA potential compared to slaughterhouse wastewater, paper mill wastewater, winery wastewater, crude glycerol, sewage sludge, and meat and bone meal. Further investigation is necessary to obtain optimum substrate composition ranges for AF.

The feedstock composition can be modified by mixing different substrates, in which case the process is known as co-digestion or co-fermentation. Co-fed substrates are known to improve yields due to synergistic effects (Feng et al. 2011). It can also help dilute toxic compounds present in the feedstocks and improve nutrients ratio, e.g. adding protein substrates improves N content. Hong and Haiyun optimized the co-fermentation of food waste and excess sludge, obtaining maximum VFA concentration of 29 g L\(^{-1}\) for 88% (VSS basis) food waste (Hong and Haiyun 2010). The synthetic food waste used in this study did not contain animal products, indicating that a certain amount of protein derived from the excess sludge was beneficial. In a different study using WAS, potato peel waste and food waste, it was found that carbon-rich substrates promoted butyrate production, whereas more proteinaceous feedstocks led towards propionate and valerate, with acetate being the predominant VFA in all cases (Ma et al. 2017). The highest VFA yield observed in this study, 344 mg COD g\(^{-1}\) VS, corresponded to a ratio of WAS to potato peel of 1:3 (in terms of VS).

Using the data presented in Table 3, a principal component analysis (PCA) was performed to elicit a more comprehensive assessment of the current literature considering both AF operating parameters and fermentation performance. The analysis only used references where data for all variables investigated was present. The first four PCs can collectively explain 72.5% of the variability in the data. The data set used for this analysis is available (Outram and Ramos Suarez 2020).

Feedstock has to be treated as a qualitative parameter. If a breakdown of feedstock components was often provided, either elemental or more simplified such as carbohydrate, protein, fat etc. then this could be added to the analysis as a quantitative variable; although this would need to be consistently reported across all literature. The feedstock’s in Table 3 were simplified into six main categories, sludge, food waste (including MSW), high protein, industrial, lignocellulosic and co-fed, i.e. mixed feedstocks. Assessment of the PC plots demonstrated clustering based on feedstock type, with similar patterns occurring between similar feedstock types e.g. food waste and high protein or sludge and co-fed (which in most cases partially consisted of sludge), Fig. 6.

The clustering is evident when considering the VFA concentration, Fig. 7. High protein wastes, including food waste, produce a higher VFA concentration and yield than other feedstock types. They have the potential to release high concentrations of ammonia, which can inhibit organisms that degrade VFAs, therefore a build-up of VFAs is observed (Placidio and Zhang 2018b). This analysis demonstrates that some feedstock types are more suited to VFA production than others.
When considering the impact of each variable on the individual PCs, Fig. 8, it can be observed that all three PCs have a high dependency on a single acid concentration. Therefore, the acid percentage distribution by feedstock type was considered, Fig. 9. In the majority of fermentations acetic acid was the main, or second, most abundant acid, hence most of the data points are in the bottom left quadrant in Fig. 9. Sludge-based AF produced mainly HAc, with a small proportion of HPr. In comparison, high protein and food waste feedstock’s produced more HBu than HPr. Industrial wastes, such as wood mill effluent, and lignocellulosic feedstocks had a tendency towards HPr production. This demonstrates the feedstock influences the final product composition.

3.3.9 Developing AF

The knowledge gained in AF parameters is highly dependent on feedstock type. Food waste is the most widely studied feedstock, followed by primary and waste activated sludges. The optimum parameters of AF towards VFA production for each substrate have not been established. Although the production of microbial VFA has proven feasible, the process is not as well understood especially compared to AD. To move forward, more optimization studies using both batch and continuous systems are necessary. As main variables are not independent, Arslan et al. (2013), and Hong and Haiyun (2010) suggest the use of statistical design of experiments (DoE) to optimize the VFA production and to understand the parameters interactions and influence.

Additionally, VFAs are inhibitory compounds, although inhibitory concentrations are generally
reported towards hydrogen and methane production, with propionate being the most toxic. In pure-culture acidogenic fermentations, propionate can be inhibitory at low concentrations of 10 g L\(^{-1}\) (Bhatia and Yang 2017). Veeken et al. (2000) found that at 30 g\(_{\text{VFA}}\) COD L\(^{-1}\) and pH 5 inhibits the hydrolysis rate. Establishing the exact VFA concentration thresholds is difficult as it depends on operational variables, microorganism consortium and type of VFA produced. A better understanding in this topic, could help establish the maximum potential of AF without the aid of in situ product recovery (ISPR).

3.4 Primary recovery of VFAs from AF

Due to product toxicity and possible product degradation, recovery of VFAs from the AF is of strategic importance. Lopez-Garon and Straathof (2014) have provided a detailed review on the recovery of single carboxylic acids from pure culture fermentations. Whilst the overall processing steps are likely to be similar, mixed VFA recovery is more complicated due to the mixture of acids that need to be separated if they are to be sold as chemicals. Before a separation process is chosen, the final product use of the VFA should be considered as this will influence the downstream processing. Based on the current literature, this is generally not taken into consideration. The recovery process must selectively target the VFAs over other fermentation broth components including water, and increase their concentration in the product stream (Reyhanitash et al. 2017). The energy demand, and the number of unit operations required to achieve the desired product stream, and the fermentation broth conditions should be considered when designing the recovery process.

The properties of the fermentation broth play a key role in designing a product recovery process. AF broths will generally contain suspended solids including cells; VFAs and other soluble compounds. VFAs are extracellular products and, therefore, can be found in the liquid fraction without the need for cell disruption. Many studies achieved VFA concentrations of 10–60 g COD L\(^{-1}\), Table 3. To facilitate product recovery, higher VFA concentrations are most desirable. Most AF studies were carried out at neutral or slightly acidic pH. The concentration and composition of soluble compounds is highly dependent on the substrate used, chemical addition to control pH, and the fermentation performance in terms of by-products formation. Suspended solids are generally at high concentrations when solid wastes are used as substrate. VSS from AF of primary sludge varied

![Weighting of each variable on individual principal components. Top: PC1, middle: PC2, bottom: PC3](image_url)
between 6 and 9 g L\(^{-1}\), depending on fermentation conditions (Wu et al. 2009). Properties of the fermentation broth excluding VFA concentrations are often unreported with very few exceptions. 200 mg NH\(_4\)\(^+\)–N L\(^{-1}\) was reported in AF broth from WAS (Meng-meng et al. 2009). Nitrogen and phosphorus concentrations fell in the ranges of 50–300 mg NH\(_4\)\(^+\)–N L\(^{-1}\) and 20–50 mg PO\(_4\)\(^{3-}\)–P L\(^{-1}\) respectively. 100–2000 mg NH\(_4\)\(^+\)–N L\(^{-1}\) and were observed in broths from food waste and kitchen wastes (Zhang et al. 2005; Jiang et al. 2013; Wang et al. 2014). As these concentrations are low, they have low probability of affecting the recovery process. Other ions such as Na\(^+\), which are present as a result of NaOH addition for pH regulation, are more likely to have an impact as they can be found at concentrations of up to 25 g L\(^{-1}\) (Zhang et al. 2005).

This section discusses all the recovery techniques that have been researched with regards to mixed VFA production from AF. The focus is on the primary separation integrated with the fermentation step for ISPR to relieve product inhibition and enable a continuous fermentation for improved VFA yields. The key factors for recovery selection and integration for each technique are collated in Table 4.

### 3.4.1 Adsorption

Adsorption is a common technique used for product recovery from bioprocesses. It relies on the formation of a bond between the target compound and a selective adsorption resin. A desorption step is required to recover the VFA and regenerate the adsorbent for further use.

Cabrera-Rodríguez et al. (2017a), Rebecchi et al. (2016), Reyhanitash et al. (2017) and Yousuf et al. (2016) all investigated adsorption of VFA from mixed culture fermentations. Unlike other product recovery techniques, there is no consensus over the mechanism of separation and pH conditions for optimal recovery, as this is dependent on the adsorbent used. Resins bind with the acids via ion exchange, when the acids are in the dissociated form (pH > pKa), or hydrophobic interactions when in the free acid form (pH < pKa) (Cabrera-Rodriguez et al. 2017a). This increases the flexibility of integrating adsorption with AF, as an appropriate adsorbent can be selected depending on fermentation conditions. All adsorption investigations focused on amine-based anion exchange resins. The use of an amine as a conjugation ion is similar to liquid–liquid extraction, where amine-based extrac-tants (e.g. trioctylamine) are used for carboxylic acid recovery (Kertes et al. 2009). Rebecchi et al. (2016) found that the Amberlyst A21 (tertiary amine) had the best adsorption performance (based on adsorption yield and solid phase VFA concentration) in grape pomace digestate, although an adsorption yield of only 11% was achieved. Amberlyst A21 being 3.5–47 times cheaper than the other resins tested also helped its favorability (Rebecchi et al. 2016). Reyhanitash et al. (2017) found the non-functionalized resin (Lewatit VP OC 1064 MD PH) to have the highest VFA adsorbing capacities, relying on the hydrophobic interactions. The more hydrophobic the VFA, the higher selectivity with the adsorbent was observed. This higher capacity could also be related to the non-functional resin not adsorbing mineral acids formed in the fermentation broth, unlike with the functionalized resins.
Table 4  Overview of reported VFA recovery techniques and the key requirements for successful separation

| Technique            | Separation mechanism | pH requirement | Selectivity<sup>a</sup> | Recovery pretreatment performed | Recovered VFA form | Other broth components removed? | References                                                                 |
|----------------------|----------------------|----------------|--------------------------|---------------------------------|-------------------|---------------------------------|---------------------------------------------------------------------------|
| Adsorption           | Electrostatic/affinity| pH > pKa<sup>b</sup> | HBu > HAc                | Clarification (typically centrifugation followed by ultrafiltration at 0.2 μm) | Depends on desorption step | Other anions                    | (Yousuf et al. 2016; Recbecchi et al. 2016; Cabrera-Rodrı́guez et al. 2017a; Reyhanitash et al. 2017) |
| Distillation         | Evaporation          | –              | HBu > HAc                | Biomass removal                 | Acid<sub>aq</sub> | Unreported                      | (Mumtaz et al. 2008)                                                     |
| Electrocoagulation   | Electrostatic        | pH > pKa<sup>c</sup> | HAc > HBu                | None                            | Acid salt<sub>aq</sub> | Yes<sup>d</sup>                | (Fayad et al. 2017)                                                      |
| Electrodialysis      | Electrostatic        | pH > pKa<sup>c</sup> | HAc > HBu                | Clarification                   | Acid salt<sub>aq</sub> | Other anions                    | (Jones et al. 2015, 2017; Zhang and Angelidaki 2015; Scoma et al. 2016; Tao et al. 2016; Arslan et al. 2017; Domingos et al. 2018; Pan et al. 2018) |
| Esterification       | Reactive             | pH < pKa<sup>c</sup> | HVa > HAc                | Clarification                   | Ester              | Unreported                      | (Plácido and Zhang 2018a)                                                |
| Filtration           | Size                 | Neutral<sup>f</sup> | n/a                      | Clarification                   | Acid<sub>aq</sub> | High possibility, unreported    | (Zacharof and Lovitt 2014; Longo et al. 2015; Jung et al. 2015; Xiong et al. 2015; Trad et al. 2015; Zacharof et al. 2016) |
| Freeze/Thaw          | Physical             | Fermentation    | None                      | None<sup>e</sup>               | Acid<sub>aq</sub> | Yes<sup>f</sup>                 | (Omar et al. 2009)                                                      |
| Gas stripping        | Evaporation          | pH < pKa         | HBu > HAc                | None                            | Acid salt          | Unreported                      | (Li et al. 2015)                                                        |
| Liquid–liquid extraction | Affinity/ Reactive | pH < pKa         | HBu > HAc                | Clarification                   | Acid complex<sub>x</sub> | Other anions                    | (Mostafa 1999; Alkaya et al. 2009; Reyhanitash et al. 2016; Bekatorou et al. 2016; Rocha et al. 2017) |
| Membrane extraction  | Affinity/ Reactive   | pH < pKa         | HBu > HAc                | Solid removal (e.g. coarse (1–2 mm) filtration) | Extractant dependent | Unreported, high possibility (extractant dependent) | (Yesil et al. 2014; Tugtas 2014; Aydin et al. 2018; Plácido and Zhang 2018a; Outram and Zhang 2018; Dessì et al. 2020) |
| Pervaporation        | Affinity/ evaporation| pH < pKa         | HBu > HAc                | Clarification                   | Acid<sub>aq</sub> | Unreported                      | (Choudhari et al. 2015; Yesil et al. 2018)                                |
| Salting-out          | Affinity            | pH < pKa         | HBu > HAc                | Clarification                   | Acid<sub>org</sub> | Unreported                      | (Fu et al. 2015, 2017)                                                    |

<sup>a</sup>Selectivity focuses on two main acids produced in Table 3, generally if HBu > HAc then selective towards longer chain VFAs and vice versa

<sup>b</sup>Generally, although if separation is primarily affinity based then pH < pKa is required (Yousuf et al. 2016)

<sup>c</sup>Needed in salt form, separation hindered if pH is too high

<sup>d</sup>Electrocoagulation removes solids from broth with VFA remaining, likely that other soluble components remain with VFA

<sup>e</sup>Acid<sub>aq</sub> used as acid catalyst (Plácido and Zhang 2018a)

<sup>f</sup>Depends on membrane and desired process operation – Xiong et al. (Xiong et al. 2015) required pH 3 whereas Jung et al. (Jung et al. 2015) used pH 8

<sup>g</sup>Freeze/thaw is a method to remove suspended solids from the fermentation broth, with a concentrating effect
(Reyhanitash et al. 2017). Yousuf et al. (2016) used a weak anion resin, Amberlite IRA-67 and granulated activated carbon Norit type Darco. In contrast to the other research, Yousuf et al. (2016) found that a pH below the pKa was beneficial for both investigated resins, with both resins offering significant acid recovery yields of 74% for Amberlite IRA-67 and 63% for the activated carbon, at 200 g adsorbent L\(^{-1}\) broth. This is due to the hydrophobic interactions dominating over the ion-exchange interactions.

Due to the wide range of resins with varying interaction types, it should be possible to find a resin to match the optimum fermentation conditions or vice versa. For example, with Amberlyst A21 and grape pomace AF pH 6.5 was best for recovery (Rebecchi et al. 2016). This is because the pH affects the concentration of active protonated amino groups on this resin, so as the pH increases the concentration of pronated amino groups decreases thereby decreasing the number of sites available for adsorption of the VFA (Rebecchi et al. 2016). Co-optimization of the fermentation and adsorption step would be beneficial.

The other key step associated with adsorption is desorption. This was not considered by Yousuf et al. (2016). Rebecchi et al. (2016) decided upon solvent based desorption from the Amberlyst A21 resin. Ethanol demonstrated VFAs desorption rates of approximately 67%, and desorption of longer chain VFAs to be higher. Whereas water, as the solvent, had higher selectivity for shorter chain VFAs. Rebecchi et al. (2016) advocate ethanol as the best solvent since it is easier to recover the VFA through distillation due to lower boiling point compared to water. This also regenerates the ethanol for reuse. VFA desorption was further improved through the addition of 1 M NaOH to the ethanol or water. This is not surprising, as NaOH is a strong base, therefore can outcompete the amine-acid bond. This is similar to the back-extraction of acids into NaOH with membrane extraction (Rebecchi et al. 2016). Despite the improvement with the use of NaOH, the end use of the VFA needs to be considered. The addition of NaOH results in the VFAs being present as sodium salts. To achieve a free acid product, then acidification using a mineral acid is required (López-Garzón and Straathof 2014). Reyhanitash et al. (2017) decided upon nitrogen stripping at elevated temperatures (180 °C) for desorption. This desorption process could produce VFA purities of up to 91 wt% from a 0.25 wt% synthetic wastewater solution.

For Cabrera-Rodríguez et al. (2015, 2017b, 2017a) the desorption step was the main focus of their research. They developed a novel CO\(_2\)-expanded alcohol desorption process to allow the direct conversion of VFAs to esters. This research is one of only a few examples where the end product use has been considered prior to VFA recovery development. The conversion to esters was quicker for the shorter chain VFAs, with Cabrera-Rodríguez et al (2017a) suggesting that there was a steric factor involved.

3.4.2 Distillation

Distillation is a widely used separation method; separating liquids based on differences in boiling point/volatility. Mumtaz et al. (2008) proposed a two-stage distillation process for the recovery of mixed VFAs from AF of POME. The first stage is to dewater the fermentation broth to achieve a VFA concentrate which is acidified using sulphuric acid then distilled further to recover the VFAs in the distillate. Prior to the initial distillation, filtration of the broth is required to allow biomass recycling as the high temperatures (>105 °C) will kill the microorganisms. The broth contained 10 g VFA L\(^{-1}\) was concentrated to 55 g VFA L\(^{-1}\) after the two-stage distillation process. This is still a relatively low concentration, as it is equivalent to the ones achieved in some fermentation processes. The biggest problem with implementing distillation is that water is the major fermentation broth component with a lower boiling point than the VFAs, Table 1. This makes distillation an energy intensive recovery process. Unless the concentration can be significantly improved, or heat integration is possible, this energy demand plus the addition of another concentration step will likely render distillation technology unfavorable for VFAs recovery.

3.4.3 Electrocoagulation

Fayad et al. (2017) proposed electrocoagulation as a primary separation method for the purification of VFAs from digestate or AF. Electrocoagulation works by supplying a current to sacrificial electrodes, e.g. aluminium or iron, which produce metal ions to induce flocculation of organic matter and nutrients. This is, therefore, predominantly a means of solid and contaminant removal and should be considered as an alternative to filtration or centrifugation. Fayad et al.
(2017) demonstrated VFAs are not flocculated in this process, remaining in solution at a constant concentration, requiring further recovery. From a glucose fermentation this technology could remove >80% of solids, nitrogenous and phosphorous species from the fermentation broth. They also demonstrate this method has the potential to be more cost effective than electrodialysis and nanofiltration (Fayad et al. 2017), but electrocoagulation does not concentrate the VFA solution.

3.4.4 Electrodialysis

Electrodialysis (ED) is the most researched separation technique for VFA recovery, with many different operating methods. It is often proposed as it requires the VFAs to be in their charged form, therefore can operate at typical fermentation pH (Table 3). A series of charged membranes are positioned between two electrodes, and a current is applied across them to facilitate ion transfer creating a VFA-rich (concentrate) chamber (Jones et al. 2015). Huang et al. (2007) provide a detailed overview of electrodialysis for the recovery of organic acids.

Conventional (CED) is the most researched ED method for VFA recovery (Jones et al. 2015, 2017; Scoma et al. 2016; Tao et al. 2016; Domingos et al. 2018; Pan et al. 2018). All of the research demonstrates that CED can remove VFAs from the fermentation broth, with 95% VFA recovery achieved by Jones et al. (2015) and 92% by Tao et al. (2016). CED also demonstrated a preference towards acetic acid recovery compared to other VFAs. The general order of selectivity was HAc > HPr > HBu > iHBu > HVa > HHex (Jones et al. 2015; Scoma et al. 2016; Pan et al. 2018). This would be advantageous if acetic acid or other smaller chain VFAs were the desired product. The benefit of this would be that the longer VFAs could be fed back to the reactor for degradation to acetic acid, then removed.

Various increases in the recovered VFA concentration, due to ED, are reported: Tao et al. (2016) stated that the VFA concentration increased from 11.73 g L−1 to 19.82 g L−1, which was in agreement with Scoma et al. (2016) who reported a concentration increase by a factor of 1.2–1.5. Pan et al. (2018) reported a 3–4 fold increase in VFA concentration. This bigger increase is due to the lower initial concentration of VFA in the feed chamber, but the overall VFA concentration in the product/concentrate chamber only reached approximately 6 g L−1 in nearly 550 h of operation. In practice, these concentrations <2 wt% are too low to use the VFA as a feedstock for other fermentation processes, concentrations of 200–500 g L−1 would be desirable.

Electrodialysis with bipolar membranes (EDBM) systems have also been tested for VFA recovery. Zhang and Angelidaki (2015) have proposed a microbial bipolar electrodialysis cell (MBEDC); the anode utilizes a biofilm to convert organic compounds into H+ ions and carbon dioxide, reducing the electricity use compared to alternative ED systems. Arslan et al. (2017) proposed a more traditional EDBM system; they suggest one of the advantages of using bipolar membranes is that the alkalinity/pH in the system can be controlled by supplying hydroxide ions to the fermentation. The VFA recovery performance of these systems was similar to CED, with Zhang and Angelidaki (2015) achieving 95% VFA recovery and Arslan et al. (2017) achieving 69%. The disadvantage of bipolar membrane systems is the cost associated with membrane maintenance and replacement (Arslan et al. 2017).

A major disadvantage with all ED processes is the preferential removal of non-VFA anions, such as Cl−. They are smaller than the VFA anions, therefore faster transfer into the concentrate is observed (Zhang and Angelidaki 2015; Scoma et al. 2016; Tao et al. 2016; Arslan et al. 2017; Domingos et al. 2018). Additionally, many ED processes require additional salts, typically sodium chloride, to be added to improve the concentration gradients for mass transfer (Zhang and Angelidaki 2015). This practice will not only complicate further downstream processing, but it may make this stream unsuitable as a fermentation feedstock, depending on the secondary fermentation microorganisms’ tolerance to these ions.

In the majority of research performed, highly clarified fermentation broths are used with the ED system. Generally, the broth is centrifuged/screened then filtered before ED (Jones et al. 2015; Tao et al. 2016; Domingos et al. 2018). This is because fouling of the membranes is reported to increase electrical resistance (Jones et al. 2015). Additionally, although it is suggested that the process is in a position to be integrated with fermentation for solids/effluent recycling (i.e. ISPR), none of the research has demonstrated this. Instead, a batch fermentation is performed.
followed by batch clarification and batch ED (Tao et al. 2016; Domingos et al. 2018).

3.4.5 Esterification

Ester production is one of the major uses of VFAs. If the esterification occurs as part of the separation process, it could increase the product routes from waste substrates. Plácido and Zhang (2018a) investigated the conversion of VFAs from fermentation broth into methyl esters, and proposed that ammonium sulphate could be produced as a byproduct due to acidification of the broth using sulphuric acid, which could increase the plant profitability. The dilute VFA concentration in the fermentation broth (100 g VFA L\(^{-1}\)) would, however, hinder the direct esterification process. For a VFA concentration of 500 g VFA L\(^{-1}\), the methyl VFA yield was less than 10%. Yields of 50% were possible with a VFA concentration of 80% (800 g VFA L\(^{-1}\)). At these yields, an organic phase formed which would improve the separation and recovery of esters from the fermentation broth (Plácido and Zhang 2018a). This would require a considerable concentration step prior to esterification; therefore esterification is more suited as a secondary downstream step rather than a primary separation step.

3.4.6 Filtration

The integration of filtration with AF is one of the most researched separation methods. There are two motives for this: solid separation (prior to further VFA recovery) and selective separation of VFA based on molecular size.

For selective VFA separation, nanofiltration (NF) is proposed, although the conclusions of this research are mixed. Zacharof and Lovitt (2014) found that the VFAs remained in the retentate, with retention ratios up to 75%. This enabled the concentration of acetic acid and butyric acid to increase by a factor of 2.5 and 1.8 respectively. The pH was found to influence the flux, productivity and retention, with optimum conditions at pH 7. In reality, this is a concentration step, and a solid removal step is required prior to nanofiltration to achieve a concentrated VFA solution in the retentate. Other soluble compounds are likely to remain, and these impurities could limit the final VFA product options. In contrast, Xiong et al. (2015) optimized the nanofiltration step to reject sugars (>90%) with 0–40% rejection of VFAs, except for butyric acid where 100% was rejected. When they integrated this process with AD, the acid concentration in the reactor was reduced by nearly 90%, meaning that 86% of the acids produced were recovered. Operation of nanofiltration in this mode would be easier to integrate with the fermentation, as the retentate can be recycled back to the reactor for further COD reduction. Unfortunately, no information on the permeate concentrations was reported. It must be noted that, similar to Zacharof and Lovitt (2014), Xiong et al. (2015) centrifuged and filtered the fermentation broth prior to nanofiltration to remove solids and reduce fouling of the membrane. Therefore, this nanofiltration process should be considered as a VFA purification step rather than VFA recovery.

Jung et al. (2015) propose using forward osmosis (FO), whereby a concentration gradient is established using a concentrated salt draw solution. This process cannot be considered a true VFA recovery process (for ISPR) as the VFAs remain in the retentate and the water is transferred to the draw solution, similar to the nanofiltration by Zacharof and Lovitt (2014). With this FO process, there was a 97% VFA rejection and the concentration was increased to 49.28 g\(_{\text{VFA}}\) L\(^{-1}\) from 35.12 g\(_{\text{VFA}}\) L\(^{-1}\) in the fermentation broth (a factor of 1.4), at an optimum pH of 8. A techno-economic comparison between nanofiltration and FO operation is required, to see if there are any justifiable benefits, as the added requirement of a draw solution and regeneration of the draw solution will increase operating costs.

3.4.7 Freeze/thaw separation

Freezing followed by thawing is a physical separation method proposed by Omar et al. (2009) as an initial step in the recovery of organic acids from AF. They froze the fermentation broth overnight at \(-30^\circ\text{C}\), followed by thawing at 60 °C for 2–3 h on a cloth mesh with a 1 mm pore size. They removed 66% of the suspended solids and saw the acid concentration increase from 59 g L\(^{-1}\) to 70 g L\(^{-1}\), an increase of 16%. Further processing steps were then applied (centrifugation, filtration and evaporation) to further concentrate the acids. The freeze–thaw process, however, is effectively a solid removal step, as other solubles such as nitrogen-based compounds were not removed from the broth (Omar et al. 2009).
3.4.8 Gas stripping

Gas stripping involves sparging a gas through the fermentation broth to transfer volatile products from the liquid phase to the gas phase, from which they can be recovered. For gas stripping to be effective, the VFAs must be present in their undissociated form. Li et al. (2015) achieved this by operating the fermentation below pH 4.8 (average VFA pKa). They conditioned their inoculum to operate under acidic pH, by dosing the anaerobic sludge with glucose to facilitate rapid VFA accumulation. The gas stripping was cyclic, only occurring when the pH was reduced below 4.8; ensuring that more than 50% of the VFAs were present in the undissociated form. The headspace gas, predominantly nitrogen, was circulated through the fermentation broth and a calcium carbonate slurry to capture the VFAs. The pH of the fermentation broth increased, indicating removal of VFA from the fermentation broth. The analysis of the liquid portion of the calcium carbonate slurry confirmed that VFA salts were present, with butyric acid being the most abundant (Li et al. 2015). This initial assessment of gas stripping for VFA recovery has positive results, but similarly to the majority of recovery techniques, it relies on the pH being less than the pKa (Table 4).

Whilst Li et al. (2015) achieved this through conditioning the fermentation to operate below pH 4.8 this is not a typical operating pH for AF, Table 3. Non-reactive extractants have also been investigated. Bekatorou et al. (2016) considered a selection of organic solvents, in particular alcohols. This would allow for direct esterification of the VFAs without the need for a second step to remove the acid from the extractant. This is an interesting approach; generally, the extractant choice is an organic solvent similar to the solute e.g. alcohols to recover alcohols from the aqueous phase (Rocha et al. 2017). As expected, the pH of the VFA solution had a significant effect on the recovery efficiency. Bekatorou et al. (2016) suggested the use of 2-pentanol, which had the best performance achieving 85% VFA recovery; or 1-butanol, which, although had a lower recovery percentage (65–78%), can be biologically produced enabling truly biobased esters.

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Rocha et al. (2017) suggested using MCFAs such as hexanoic, octanoic and decanoic acid as non-reactive extractants. As MCFAs are partially soluble in water, an inexpensive diluent such as toluene or n-hexane is required to facilitate extraction. The MCFAs present both hydrogen bond donors and acceptors; therefore, have the potential to address the acid functionality of the VFAs. One of the key factors in this research was diluent selection, as n-hexane decreased the number of dimerization sites between the MCFA and VFA, therefore reduced the extraction potential. The other key outcome was that the addition of an MCFA extractant did not have a linear effect on the % recovery of VFA; pure hexane recovered 6% of the butyric acid, but 5% hexanoic acid in hexane resulted in 51% recovery. Further increases to 55% and 100% ratio, extraction temperature and mixing rate were optimized. Alkaya et al. (2009) also used TOPO in kerosene, but focused on the influence of pH on recovery. At pH 2.5, the total VFA recovery efficiency was 67% with 20% TOPO in kerosene, at pH 5.5 this decreased to 32% due to less VFA being available in the free acid form. Reyhanitash et al. (2016) investigated the use of trioctylamine (TOA) in n-octanol, and several phosphonium ionic liquids as possible extractants. pH was, again, a controlling factor in VFA recovery for both these extractants. It is well-established that for affinity based separation techniques such as LLE the acid is required to be in its protonated form, therefore requiring the pH to be lower than the pKa of the acid. This is a significant problem for AF, where the fermentation pH tends to be higher than the pKa.

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hexanoic acid resulted in 80% and 85% recovery, respectively. This non-linear relationship could be beneficial to developing a recovery process with as low as possible economic footprint (Rocha et al. 2017). This is similar to the results observed by Alkaya et al. (2009) using TOPO in kerosene.

Ionic liquids (ILs) have been considered as an alternative to organic extractants. They have been shown to have higher distribution coefficients (the ratio of acid between the extractant phase and aqueous phase at equilibrium) for carboxylic acids at low concentrations, therefore ideal for the concentration ranges obtained via AF. Additionally their high boiling and decomposition temperatures allow for regeneration by evaporative methods (Reyhanitash et al. 2018). Reyhanitash et al. (2016) demonstrated that ILs, in particular trihexyl(tetradecyl)phosphonium bis-2,4,4-(trimethylpentyl) phosphinate ([P$_{666,14}$][Phos]) could produce a more concentrated VFA stream, 62.9 wt% total VFA, compared to 50.2 wt% total VFA with 20 wt% TOA in n-octanol, which would allow for lower solvent to feed ratios. Similar to organic extractants, the mechanism of recovery is primarily hydrogen bonding, requiring the VFA to be in their free acid form (Reyhanitash et al. 2016). The use of ILs as an extractant for VFA recovery has only been tested with synthetic VFA mixtures, rather than real AF broth. Reyhanitash et al. (2016) considered the impact of different salts present in waste water on the extraction process, which influence the choice of IL. The addition of salts caused a reduction in the distribution coefficient of acetic acid between the broth and extractant, with all extractants. It is believed that the salt anions form complexes with the extractant reducing the availability for extraction of the acid. This observation was also made with 20 wt% TOA in n-octanol. Reyhanitash et al. (2016) observed that careful selection of the IL anion was required. Cl$^-$ and Br$^-$ anions were found to leach significantly into the fermentation broth, resulting in an ion exchange mechanism for extraction of the VFA. This results in an irreversible extraction, as the VFA cannot be recovered without replacing the leached Cl$^-$ or Br$^-$ ions. Anions such as phosphate did not significantly leach into the fermentation broth, therefore are a better extractant. IL regeneration methods have been studied by Reyhanitash et al. (2019), based on an acetic acid concentrations of 1 wt% in the broth, the back extraction with a volatile base such as ammonia or trimethylamine allowing for recovery of the free acid reducing further downstream processing. They were able to produce a final product stream with 90 wt% acetic acid (10 wt% water). The impact of real fermentation broth on the IL and the subsequent regeneration needs to be considered.

Overall, LLE appears to be a promising recovery technique. All LLE research indicates: that pH < pKa is beneficial; selectivity is higher for longer chain acids, although recovery of acetic acid is possible; and a larger ratio of extractant to diluent is more effective, but less economically favorable. Most of the research using real fermentation broth required a clarification step (filtration or centrifugation) prior to extraction, research into the necessity of this step is advised to understand the scale up requirements. Additionally, existing research does not, typically, comment on the concentration of VFA in the extractant or how to recover the VFA from it. Reyhanitash et al. (2018) have performed an initial review and process assessment of extractant regeneration, indicating that all methods require more energy for separation than the heat of combustion for VFAs potentially limiting the industrial applicability based on the current developmental states.

3.4.10 Membrane extraction

Membrane extraction is an extension of LLE, whereby a membrane is placed between the aqueous feed and extractant. Typically, to recover carboxylic acids, membrane extraction utilizes a hydrophobic porous membrane with an organic extractant such as TOPO or TOA in a diluent (Solichien et al. 1995; Vajda et al. 2003). Once the extractant is acid-rich, it is passed across a second membrane extraction unit, where a concentrated sodium hydroxide stripping solution is used to regenerate the extractant and return the carboxylic acid to the aqueous phase (Schlosser et al. 2005). To achieve the carboxylic acid as a free acid in the aqueous phase, the VFA-sodium hydroxide solution would need to be re-acidified with a mineral acid, such as sulphuric acid. This will produce a mineral salt that will need to be disposed of or found a use for (López-Garzón and Straathof 2014).

For recovery of VFA, Plácido and Zhang (2018a) followed the traditional method of membrane assisted extraction using a polypropylene porous membrane with TOA in 1-octanol. They achieved 80% recovery
of all acids other than acetic acid, from acidified fermentation broth, in 24 h of operation. This could lead to selective recovery of VFA, which could be beneficial, depending on the final product use. The downside to this work is that the fermentation broth needed to be acidified prior to membrane extraction; this is the same as other affinity separation techniques.

Alternatively the organic extraction step can be eliminated, and a sodium hydroxide solution is used as the extractant. A hydrophobic polytetrafluoroethylene (PTFE) membrane separates the two aqueous phases, with the pores remaining air-filled to ensure separation of only the volatile products. Acidification of the fermentation broth from pH 6.6 to 3 saw a 65 times increase in the overall mass transfer coefficient achieved for butyric acid (Yesil et al. 2014; Tugtas 2014). Aydin et al. (2018) found that there was no selective recovery with air-filled pores and established that filling the pores with an organic extractant increases the selectivity for VFA. There are still questions, however, as to the long-term stability and operation of utilizing an extractant-filled membrane.

These membrane extraction methods result in multiple downstream processing steps, with high mineral acid and base consumption rates to transfer the VFAs to the final aqueous phase. To reduce the downstream processing steps Outram and Zhang (2018) demonstrated that a non-porous silicone membrane with a water extractant can selectively recover larger chain VFAs, from an acidified fermentation broth. Using water as the extractant, means that the VFAs are present in an aqueous solution, therefore reducing the number of downstream processing steps. The direct use of a real fermentation broth, without any large (>1 mm) solids removal step, did not impede product recovery as improved mass transfer coefficients were observed compared to a synthetic VFA solution. The use of a non-porous membrane hindered the mass transfer compared to porous membrane alternatives with a sodium hydroxide extractant. Therefore, a larger membrane area is required for the same VFA flux, but this is offset against the negligible extractant cost and decreased downstream processing. Dessì et al (2020) applied this method to a cheese whey dark fermentation, and was able to recover butyric acid achieving concentrations of 2.5 g L\(^{-1}\) and greater than 90% purity of the recovered products (pH 5). The high purity reduced as fermentation broth pH decreased to 3, but sugars, nutrients, and lactic acid did not cross the membrane therefore facilitating an easier downstream recovery.

### 3.4.11 Pervaporation

Pervaporation separates volatile components across a membrane which is subjected to a vacuum, or sweep gas, on the permeate side. The membrane material selectively separates the products based on diffusion. Choudhari et al. (2015) developed a novel polyether block amide (PEBA) graphene composite membrane for pervaporation, with focusing on butyric acid recovery. The pervaporation unit was tested on digestate taken from AD of perennial grass and dairy wastewater. Like many other techniques, the broth was microfiltered followed by an ultrafiltration step (10,000 MWCO), then acidified to pH 3.7. Even with this degree of solids removal, a decrease in flux was observed compared to pervaporation of a synthetic broth. Results showed that the butyric acid concentration could be increased from 6 g L\(^{-1}\) to 114 g L\(^{-1}\) (19 times increase). When considering the separation factor (ratio of VFA and water in the permeate divided by the ratio of VFA and water in the feed), valeric acid was higher than butyric acid indicating that pervaporation with PEBA composite membranes has higher selectivity for larger, more hydrophobic VFAs.

Yesil et al. (2018) considered three membranes for the recovery of VFAs using synthetic broth: polytefluoroethylene (PTFE), PTFE filled with tridecylamine (TDDA) and a silicone-PTFE composite. They found the TDDA filled PTFE membrane to offer the highest VFA flux and separation factor. This is due to the VFAs forming a complex with the TDDA in the membrane. The separation mechanism resembles extraction more than evaporation and diffusion. VFAs have a greater affinity to water than other fermentation products such as alcohols; this will make any separation based on volatility harder. All the membranes used, except PTFE, are in the research phase of development which makes it difficult to compare with commercial pervaporation processes. Additionally, Choudhari et al. (2015) and Yesil et al. (2018) did not consider the ease of product capture (in a commercially viable method) from the vapor phase. This step could lead to significant product loss if not carefully considered or investigated.
3.4.12 Salting-out and salting-out extraction

The addition of a salt to facilitate phase separation is common practice. Fu et al. (2015, 2017) have investigated this as a method to help improve the extraction of VFAs from synthetic broth. The selection of the optimum salt-extractant combination plays a major role. Fu et al. (2015) initially investigated the separation of carboxylic acids, from a mixture containing formic, acetic, propionic, lactic, succinic and citric acids, using an ethanol-ammonium sulphate combination. This system was more effective for the extraction of the VFAs compared to acids with multiple OH groups (e.g. lactic or succinic acid), as the latter are more hydrophilic. Like LLE, decreasing the pH resulted in an increase in the partition of acid in the organic phase.

The salting-out extraction for a butyric and acetic acid mixture was also tested using monohydric alcohols with monosodium phosphate (Fu et al. 2017). It was initially shown that salting-out can induce phase separation, but the low acid concentration required high salt concentrations and the phase separation was difficult to observe. Therefore, an extractant was used to improve the separation. Earlier work had established that the free acid was required in LLE (hence pH < pKa). With monosodium phosphate more phosphate ions were present as the pH increased to 6. The higher concentration of phosphate ions increased the salting-out capability of the system, hence an increase in acid separation was observed. This could potentially allow VFAs recovery without the need for acidification.

These examples of salting-out extraction, have used alcohols as the extractant. The salt addition improves the capability of the extractant, and reduces the degree of miscibility of the small alcohols (e.g. ethanol) in water. Both Fu et al. (2017) and Yan et al. (2018) considered a selection of low molecular weight alcohols. They demonstrated that as the hydrophobicity of the alcohol increased, the distribution of extracted acid decreased. Fu et al. (2017) achieved their highest distribution coefficient, 106, with an ethanol-monosodium phosphate system. For a 50 g L\(^{-1}\) butyric acid solution, with the addition of 23 wt% monosodium phosphate, 94% of the butyric acid was recovered.

The application of salting-out extraction for VFAs recovery is still in its very early stages. The next step is to apply it to VFA-rich fermentation broth. A salt concentration of 23 wt% is likely to be toxic to the bacteria used in AF, limiting direct integration with the fermentation broth for ISPR. External application could be a solution, but a salt recovery process will be required to minimize the amount of salt which is returned to the fermenter for further processing and reduce the cost of this unit operation.

3.4.13 Recovery technique comparison

As the recovery techniques have been developed independently of the fermentation, many of them require different conditions, Table 4, compared to the fermentation, Table 3. The two major factors slowing down the implementation of VFA ISPR are the degree of clarification and acidification required prior to recovery. Ideally, the primary recovery step should be applied directly to the fermentation broth. Many authors centrifuged and microfiltered the broth prior to the VFA recovery technique; this level of processing is likely to be unsustainable for scale up. Additionally, many authors adjust the pH prior to recovery through acidification to successfully recover VFAs, Table 4, but no consideration is made to the impact of this acidification step either technically or economically. For a continuous fermentation process it is advantageous to remove the VFAs and recycle the fermentation broth and solids back to the reactor for further processing to maximize yield, productivity, and waste destruction. For this, the broth needs to be neutralized, which will result in mineral salt formation. The impact of the acidification, neutralization and salt formation on the fermentation process needs to be investigated.

Membrane extraction, electrodialysis and filtration have been the most researched recovery techniques. It is not possible to select a single technique that should be the focus for future VFA recovery research. Selection of a recovery technique should consider: the fermentation conditions, major acid produced, ideal recovered form and end-product use. If the major fermentation acid is acetic acid then techniques which utilize the dissociated acid will have increased selectivity, such as electrodialysis. For longer chain VFAs, such as butyric acid, techniques that rely on the acid being in the undissociated form tend to be more favorable. Contemplating factors such as these in the
initial process design will enable faster, more targeted development of AF process.

3.5 Process economics

To date, there has been a very limited analysis of the economics driving the move from biogas to VFA production using waste substrates. There is a more diverse product range for VFAs than biogas, opening new markets as the search for alternatives to petrochemical-based products continues. The biggest difficulty with performing an economic analysis is the lack of defined process route. Bonk et al. (2015) overcame this by assuming a VFA sales price thereby stating the maximum permissible purification cost, which other researchers could use to justify their purification process. They assumed that the VFAs would be separated into their individual acids, creating numerous product streams. Using MSW as a feedstock resulted in a maximum purification cost of 14.96 USD m$^{-3}$ effluent with no pretreatment and 15.5 USD m$^{-3}$ effluent if pretreatment was used, due to increased VFA production. This was considered acceptable as purification cost of seawater via reverse osmosis is 1 USD m$^{-3}$ effluent, which involves the separation of water from a similar concentration of solute to VFA. Even though AF fermentation broth is more complex, the allowance for a purification cost ten times greater than seawater desalination should be satisfactory to overcome the challenges with VFA recovery. No analysis of VFA recovery and separation into individual acids could be found in the literature. The current research indicates that the production of VFAs has the potential to produce higher revenues than biogas production. Bastidas-Oyanedel and Schmidt (2018) confirm this with an economic analysis of a food waste biorefinery for the production of VFAs (in particular acetic and butyric acid) compared to the more traditional AD with biogas upgrading. They found that the production of VFAs was more profitable, with a profit of 296 USD tVS$^{-1}$, compared to 19 USD tVS$^{-1}$ for gas to grid AD. The downside to AF compared to AD was the increased operating costs for AF due to the complexity of separating the acids. This analysis provides a good starting point, encouraging researchers to diversify away from AD to VFA production. The economics will vary considerably depending on the specific process route and the eventual product market.

Many researchers assume that separation of the individual acids will not be required. Fasahati and Liu (2014) calculated that the minimum selling price of the mixed VFAs from brown algae would be 384 USD t$^{-1}$, which is lower than the market price for (petrochemical) acetic acid. Their process did not account for acidification of the fermentation broth which would be required for their selected recovery method, membrane distillation, or the final potential market of the mixed VFA solution. Liu et al (2018a) considered the production of VFAs from wastewater, to be used as the carbon source for the wastewater treatment plant’s biological nutrient removal (BNR) process. They indicate that VFA production would increase profits 2.5 times that of biogas production. The increased profitability is related to smaller capital investment costs due to the shorter residence time of AF compared to AD, higher sales value, and direct use of VFAs, therefore, no expensive downstream processing is required. Fernández-Dacosta et al. (2015) considered the economics of VFAs for PHA production. They found that, compared to equivalent petrochemical plastics such as polyethylene terephthalate, PHA is not yet economically competitive. This is surprising as it is often the preferred route for mixed VFA use; but it highlights the importance of considering the economics of a process early on in the development. The process they investigated was relatively simple and they did not require a VFA recovery stage, which would normally have a high contribution to the operating costs.

The economic analysis has highlighted the importance of understanding the marketable product generated. The use of VFAs as a feedstock for other bioprocesses does not look favorable under current research developments. This should be reassessed with continuing developments in this field, and greater integration of the two bioprocesses is required. In the context of wastewater treatment plants, using VFAs as a feedstock for BNR appears to be the most promising application in the current development landscape, due to the direct integration of the two processes with minimal intermediate treatment. If this level of integration could be considered for other bioprocesses, then it might be possible to develop waste treatment plants into biorefineries and further integration into the circular economy. This is of interest to industry as demonstrated by large EU Horizon 2020 grants, VOLATILE (European Commision 2017a) and
URBIOFIN (European Commission 2017b), representing 35 different organizations and 12 EU countries. The early signs are that VFAs from waste have the potential to compete with petrochemical VFA, but more research is required for product use, fermentation optimization and development and integration of the downstream setup.

4 Progressing AF and VFA production

Current research is focused on either fermentation development or primary product recovery, with very little interaction between the two. For AF to offer competitive bio-VFAs to the market, research needs to be tailored with more thought to the end product and integrated process design. Current research is non-specific, rather than focus on maximizing general VFA yield or concentration, researchers should have specific/strategic aims for why they want to produce VFA—e.g. as a means of waste destruction or for the production of bio-VFAs as an alternative to petrochemicals. Researchers need to consider the use of the VFA—e.g. feedstock for fermentation or as individual acids as a bulk chemical (for use in other processes). This will enable more targeted fermentation optimization and process design. Additionally, this will allow for detailed techno-economic analyses to be performed.

Fermentation research needs to consider more advanced experimental designs. Naturally, AF will have high degrees of variability, from feedstocks (location and seasonal) through to inoculum source and microbiome present in the reactor. Technologies such as –omics analysis could help provide insight into making more generalized claims of how to optimize a fermentation towards the specific goal. The design process needs to account for the inherent variability which will be encountered—e.g. understanding the ideal operation space for pH and high nitrogen feedstocks (which cause an increase in pH) to maximize VFA yield and concentration. From recovery literature it is clear that a compromise will be required on optimum fermentation and product recovery conditions. Research needs to account for this within experimental design, therefore more integration of the two steps is required at laboratory scale. The final product use should also be considered when designing the recovery method. The conditions required for any proceeding downstream unit operations could influence the selection of the primary recovery step.

5 Conclusion

It is well-established that biobased production of building block chemicals is beneficial for achieving a circular economy. The production of VFAs from waste sources or biomass from non-human consumption energy crops can help achieve this. Existing research has demonstrated the potential of acidogenic fermentation, and progress is being made for both upstream and downstream processing. Future research needs to look at whole process design, from feedstock pretreatment through fermentation and product recovery to routes into the market. This requires establishing more strategic, multidisciplinary research projects to further progress AF and bio-VFA production, and utilizing more sophisticated research and operation tools to enable a better understanding of the process and adaption to the inherent variability in the process.

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Author contributions

Maria Ramos Suarez: Conceptualization, Methodology, Writing—Original Draft, Writing—Review and Editing. Yue Zhang: Conceptualization, Writing—Review and Editing, Supervision, Funding acquisition. Victoria Outram: Conceptualization, Methodology, Writing—Original Draft, Writing—Review and Editing, Supervision.

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Availability of data and material

Data supporting this study are openly available at doi.org/10.6084/m9.figshare.11854857.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.
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