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**Potential of anaerobic co-fermentation in wastewater treatments plants**

*A review*

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Review

# Potential of anaerobic co-fermentation in wastewater treatments plants: A review

N. Perez-Esteban [a], S. Vinardell [a], C. Vidal-Antich [a,b], S. Peña-Picola [a], J.M. Chimenos [d], M. Peces [c], J. Dosta [a,b], S. Astals [a,*]

[a] Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain
[b] Water Research Institute, University of Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain
[c] Department of Chemistry and Bioscience, Centre for Microbial Communities, Aalborg University, Fredrik Bajers Vej 7H, 9220 Aalborg, Denmark
[d] Department of Materials Science and Physical Chemistry, University of Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain

## HIGHLIGHTS

- Co-fermentation to enhance short-chain carboxylic acids production in WWTPs.
- WAS is the most used main substrate and food waste is the most used co-substrate.
- pH, temperature and mixing ratio are the most researched variables.
- The lack of continuous experiments limits co-fermentation application.
- Integration of co-fermentation with other biotechnologies in WWTP is discussed.

## GRAPHICAL ABSTRACT
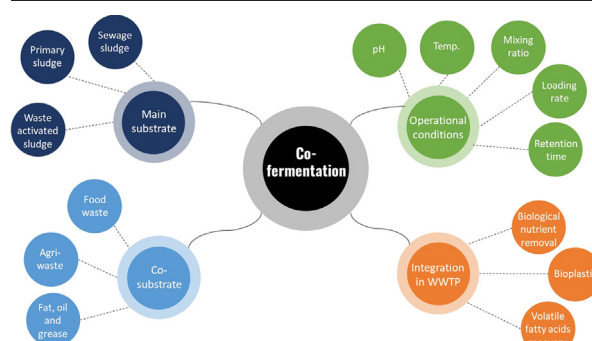


## ARTICLE INFO

## ABSTRACT

Fermentation (not anaerobic digestion) is an emerging biotechnology to transform waste into easily assimilable organic compounds such as volatile fatty acids, lactic acid and alcohols. Co-fermentation, the simultaneous fermentation of two or more waste, is an opportunity for wastewater treatment plants (WWTPs) to increase the yields of sludge mono-fermentation. Most publications have studied waste activated sludge co-fermentation with food waste or agri-industrial waste. Mixing ratio, pH and temperature are the most studied variables. The highest fermentation yields have been generally achieved in mixtures dominated by the most biodegradable substrate at circumneutral pH and mesophilic conditions. Nonetheless, most experiments have been performed in batch assays which results are driven by the capabilities of the starting microbial community and do not allow evaluating the microbial acclimation that occurs under continuous conditions. Temperature, pH, hydraulic retention time and organic load are variables that can be controlled to optimise the performance of continuous co-fermenters (i.e., favour waste hydrolysis and fermentation and limit the proliferation of methanogens). This review also discusses the integration of co-fermentation with other biotechnologies in WWTPs. Overall, this review presents a comprehensive and critical review of the achievements on co-fermentation research and lays the foundation for future research.

* Corresponding author.
E-mail address: sastals@ub.edu (S. Astals).

**Contents**

## 1. Introduction

The implementation of the circular economy paradigm requires the transition of waste treatment systems from end-of-pipe towards integrated resource recovery (Puyol et al., 2017). Resource recovery schemes conceive waste streams as a source of energy, chemicals, nutrients and water instead of as a source of pollution that needs treatment or disposal (Nghiem et al., 2017; Vinardell et al., 2020). However, the transformation of WWTPs into biorefineries advocated for decades is, to date, far from been accomplished. Pivotal to these resource recovery schemes are microbial-mediated biotechnologies capable of transforming organic waste into platform chemicals (e.g., fatty acids, methane, hydrogen), high-value products (e.g., biofuels, bioplastics, fertilizers) and clean water (Ramos-Suarez et al., 2021; Venkata Mohan et al., 2016).

Fermentation stands as an essential biotechnology in waste processing biorefineries (also referred to as resource recovery facilities (RRF)) since it allows converting organic waste into easily assimilable organic compounds such as volatile fatty acids (VFA), lactic acid and alcohols (Agler et al., 2011; Allegue et al., 2021; Dahiya et al., 2018). The conversion of organic waste into these compounds is achieved by applying selective operational conditions that suppress the methanogenesis step of the anaerobic digestion process (e.g., pH, loading rate, retention time) (Kleerebezem et al., 2015; Peces et al., 2021). For waste streams, acidogenic mixed-culture fermentation is more readily achievable than lactic and alcohol fermentation due to the complex and variable composition of the waste and the presence of inherent microorganisms in the waste. Furthermore, unlike pure-culture and co-culture fermentation, mixed-culture fermentation has limited control on the fermenters' microbial community structure and dynamics, which is an additional layer of complexity towards achieving a reliable full-scale process (Agler et al., 2011; Peces et al., 2021).

Mixed-culture fermentation liquors from waste have a relatively low market value due to their composition (mixture of fermentation products), relatively low concentration and purity (presence of other organic compounds, nutrients and salts). However, these fermentation products can be directly utilised as a carbon source to support other biotechnologies in the biorefinery such as biological nitrogen removal and recovery, biological phosphorous removal, biological sulphate reduction, bioplastics production and chain elongation, among others.

In WWTPs, primary sludge (PS) and waste activated sludge (WAS) are thickened and fed to anaerobic digesters (AD) for biogas production. However, due to their high organic content, PS and WAS (or their mixture) can be fermented to produce VFA prior AD (Bahreini et al., 2020). Fermentation is particularly attractive for WWTPs purchasing external carbon sources (e.g., methanol) to carry out biological nitrogen removal and enhanced biological phosphorus removal since the fermentation products can reduce the purchase of chemicals with a direct impact on operating costs. Indeed, the need for easily assimilable organic carbon in WWTPs is expected to increase as the nutrient discharge standards become more stringent. However, reported fermentation yields from PS and WAS are rather low. VFA yields for PS range between 19 and 300 mgCOD·gVS$^{-1}$ (Banerjee et al., 1999; Bouzas et al., 2002; Peces et al., 2016, 2020; Ucisik and Henze, 2008), while VFA yields for WAS range between 10 and 250 mgCOD·gVS$^{-1}$ (Chen et al., 2007; Guo et al., 2015; Liu et al., 2012; Ma et al., 2017; Peces et al., 2020). These fermentation yields may be sufficient to support biological nitrogen and phosphorus removal in WWTPs, however, higher fermentation yields would be required to implement more profitable biotechnologies.

Intensification of PS and WAS fermentation can be achieved at the expense of chemicals and/or energy. For instance, pre-treatments can be carried out to overcome hydrolysis rate limitations and prevent the proliferation of methanogens (Liu et al., 2009; Moretto et al., 2019). Alternatively, other waste can be fermented together with PS and WAS to increase VFA production, a process known as co-fermentation.

Co-fermentation is defined as the simultaneous fermentation of two or more substrates (Fang et al., 2020; Peces et al., 2020). Co-fermentation overcomes the limitations of single-substrate fermentation (mono-fermentation) by (i) increasing the organic loading rate (OLR), (ii) providing additional buffer capacity (prevents pH drops and alkali consumption), (iii) modifying the organic matter composition, (iv) balancing macronutrients (e.g., C/N ratio), (v) diluting potential inhibitory and toxic compounds, and/or (vi) providing an active fermentative microbial community (Banerjee et al., 1999; Fang et al., 2020; Peces et al., 2020; Wu et al., 2016). Overall, co-fermentation stands as an opportunity to make better use of the future fermentation infrastructure due to its potential to boost fermentation yields and drive the fermentation product profile without incurring into major capital and operating costs.

The authors would like to clarify that the term "anaerobic co-fermentation" and "anaerobic co-digestion" cannot be used as synonyms. The term "anaerobic co-fermentation" refers to processes aiming to promote the accumulation of low molecular weight organic molecules (e.g., VFA, lactic acid, ethanol) by limiting the proliferation of methanogenic archaea. On the other hand, the term "anaerobic co-digestion" refers

to processes that promote the activity of methanogenic archaea for biogas production. There are several reviews in the peer-reviewed literature devoted to anaerobic co-digestion (Mata-Alvarez et al., 2014; Nghiem et al., 2017; Xie et al., 2016), dark fermentation for hydrogen production (Ghimire et al., 2015; Łukajtis et al., 2018) and mixed-culture waste mono-fermentation (Atasoy et al., 2018; Fang et al., 2020; Lee et al., 2014). However, to the best of the authors' knowledge, a literature review devoted to co-fermentation to produce short-chain carboxylic acids and alcohols is not yet available.

Co-fermentation is a rather new concept with publications devoted to the topic being noticeable from 2013 onwards (see publication list in Table S1 and Supplementary Fig. S1). However, (i) the transformation of waste treatment facilities, (ii) the central role of fermentation in most biorefineries schemes, (iii) the development and implementation of new and emerging biotechnologies (e.g., green manufacturing), and (iv) the implementation of new sustainable policies (e.g., green deal or zero waste) combined with the capacity of mixed-culture fermentation to handle complex and variable organic waste make (co-)fermentation an emerging and worthwhile topic of research.

Most co-fermentation publications have used WAS as the main substrate (i.e., the predominant substrate in the mixture), while PS and mixed sewage sludge (SS, i.e., mixture of PS and WAS) are the second and third most used main substrates in co-fermentation publications (Fig. 1). These outcomes show that co-fermentation is expected to have an important role in future WWTPs. Other organic-rich wastes such as food waste (FW), pig manure (PM) and agro-industrial waste (AgriW) have been rarely used as the main substrate. However, FW and AgriW are the most common co-substrates, probably due to their fast and high biodegradability. In this review, the term FW comprises a variety of substrates with similar characteristics such as organic fraction of municipal solid waste (OFMSW), biowaste, kitchen waste, canteen waste, synthetic FW and alike; while the term AgriW encompasses a wide range of organic waste generated during the production and processing of food and fodder such as sugar cane residues and palm oil mills waste. Fig. 1 includes four publications that studied the co-fermentation of WAS and PS; however, these publications are not discussed in this review since this mixture is not designed to overcome the limitations of sludge mono-fermentation.

This publication aims to present a comprehensive and critical review of the achievements and perspectives of mixed-culture co-fermentation in WWTPs. This review focuses on co-fermentation publications targeting the production of easily assimilable organic compounds (e.g., VFA, lactic acid and alcohols) in WWTPs using sludge (WAS, PS and mixed sludge) as the main substrate. Recommendations for the selection and application of different co-substrates as well as the integration with other technologies

are discussed. Finally, the review identifies a series of knowledge gaps that require further research.

## 2. Waste activated sludge co-fermentation

WAS is the most used main substrate in co-fermentation research. The most studied mixture is WAS and FW followed by mixtures between WAS and AgriW (Fig. 1). Mixture selection relies on the ability to establish strategic and integrated platforms. WAS and FW mixtures are attractive for waste-based biorefineries located near populated metropolitan areas since large and constant amounts of these wastes are produced in these regions. On the other hand, a mixture between WAS and AgriW is of interest in agropoles and agro-processing regions. Tables 1 and 2 summarise WAS-FW co-fermentation publication carried out in batch assays, while Table 3 summarises WAS-FW co-fermentation publications carried in continuous experiments.

WAS stands as an ideal main substrate for co-fermentation applications, particularly when mixed with (i) highly biodegradable organic waste with limited (or no) alkalinity (e.g., FW or crop residues) and (ii) organic waste that cannot self-sustain a microbial community (e.g., crude glycerol, lignocellulosic waste or grease trap). WAS could also be used to adjust the moisture content of waste that has a high solid concentration (Wu et al., 2016).

### 2.1. WAS and FW co-fermentation

FW is a heterogeneous highly biodegradable waste that has low alkalinity (Li et al., 2013; Strazzera et al., 2021). The benefit of co-fermenting WAS and FW relies primarily on (i) the WAS buffer capacity to sustain the pH above 5.0 and prevent fermentative bacteria severe inhibition by low pH (Zhou et al., 2018) and (ii) the high biodegradability of FW (>80%) to boost fermentation yields (Dahiya et al., 2015). Mixtures between WAS and FW have been mainly designed to balance the C/N ratio, likely due to the influence of anaerobic co-digestion literature. However, optimising mixtures based on C/N ratio is an oversimplification since it is a proxy for macronutrients availability, ammoniacal nitrogen concentration and alkalinity (if inorganic carbon is not removed prior analysis). However, it does not consider other important factors such as substrate biodegradability, the chemical nature of the carbon source, micronutrients and microbial communities, among many others. Furthermore, the optimum C/N ratio does not converge on a single value; so, it seems that the optimum C/N ratio is mixture specific. Additionally, the C/N ratio does not consider the operational parameters of the fermenters (e.g., temperature, pH, HRT or OLR) nor waste pre-treatment.

#### 2.1.1. Impact of pH on WAS-FW co-fermentation

It is well-known that pH controls the fermentation yield and product profile of mixed-culture fermentation, yet the pH itself is not enough to predict the product profile (Fang et al., 2020; Hoelzle et al., 2014; Zhou et al., 2018). Table 1 summarises the publications that have studied the impact of pH on WAS-FW co-fermentation. Feng et al. (2011, 2009) co-fermented WAS and FW at different pH (from 4 to 11 using unit increments) in batch assays at room temperature (~20 °C). In both publications, the highest co-fermentation yield was achieved at pH 8 and 9. In Feng et al. (2009) the higher yields at pH 7–9 were related to the higher activity of some metabolic enzymes under this pH range. Accordingly, the lower co-fermentation yields at pH < 6 and pH > 9 could be explained by the lower enzymatic activity of fermentative bacteria under these pH conditions. The co-fermentation yield at pH 8 was much higher than the achieved from WAS and FW mono-fermentation controls at pH 8. Feng et al. (2011) attributed these results to (i) the organic carbon and nutrients provided by FW and (ii) the alkalinity provided by WAS. The VFA profile between pH 6 and 9 in Feng et al. (2011, 2009) was dominated by propionic acid (~50%), acetic acid (~30%) and n-butyric acid (~10%). At alkaline pH (i.e., pH 10 and 11), acetic acid was the main VFA in both publications, which is in agreement with most mixed-culture fermentation publications (Cheah et al., 2019; Esteban-Gutiérrez et al., 2018; Ma et al., 2017; Owusu-
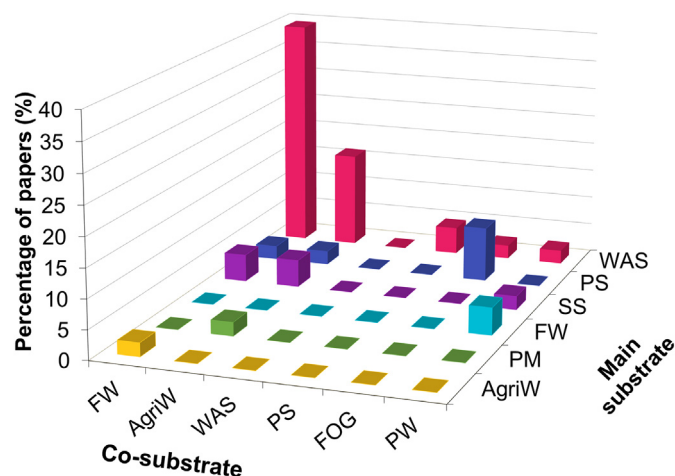


**Fig. 1.** Main substrates and co-substrates in co-fermentation publications. AgriW: agro-industrial waste, FOG: fats, oils and greases, FW: food waste, PS: primary sludge, PW: paper waste and WAS: waste activated sludge,

**Table 1**

Summary of WAS and FW co-fermentation publications studying pH and temperature in batch assays.

| Co-substrate (CoS) | T (°C) | pH control | WAS:CoS mixing ratio | $VFA_{max}$ (mg COD·L$^{-1}$) | Fermentation yield (mg COD·g VS$^{-1}$) | Main product | Reference |
|---|---|---|---|---|---|---|---|
| Canteen | 20 | No | 66:33 (%wt) | ~2000 | – | – | (Feng et al., 2011) |
| | | 4 | | 2426 | – | HAc | |
| | | 5 | | ~4000 | – | HAc | |
| | | 6 | | 5023 | – | HPro | |
| | | 7 | | 6541 | – | HPro | |
| | | 8 | | 8237 | – | HPro | |
| | | 9 | | 7912 | – | HAc | |
| | | 10 | | ~5500 | – | HPro | |
| | | 11 | | 4273 | – | HAc | |
| Canteen | 5 | 8 | 20/1 (C/N) | – | 53 | – | (Chen et al., 2013a) |
| | 35 | 4 | | – | 391 | – | |
| | | 8 | | – | 666 | HAc | |
| | | 12 | | – | 140 | – | |
| | 65 | 8 | | – | 318 | – | |
| Restaurant | 20 | 8 | 19:81 (%wt) | 2800 | – | HAc | (Li et al., 2014) |
| | 35 | | | ~9250 | – | HAc & HPro | |
| | 50 | | | 2300 | – | HAc | |
| | 65 | | | 2400 | – | HAc | |
| OFMSW | 55 | No | 65:35 (%V) | 4700 | 60 | HAc | (Moretto et al., 2019) |
| | | 7[a] | | 12,500 | 220 | HAc | |
| | | 9[a] | | 27,500 | 520 | HAc & HPro | |
| | 37 | No | | 18,600 | 290 | HAc | |
| | | 7[a] | | 27,500 | 440 | HAc | |
| | | 9[a] | | 30,000 | 490 | HAc & HPro | |
| OFMSW[b] | 37 | No | | 22,500 | 260 | HAc | |
| | | 7[a] | | 37,000 | 500 | HPro | |
| | | 9[a] | | 41,000 | 570 | HPro | |
| Canteen | 35 | 9 | 14:86 (%VSS) | ~4000 | – | HAc | (Xu et al., 2020) |

[a] The pH was only adjusted at the beginning of the experiment.
[b] Thermal pre-treatment at 72 °C during 76 h.

Agyeman et al., 2021). Interestingly, Feng et al. (2011) reported a relatively high ethanol concentration at pH 10 and 11. The test at pH 4 was fully inhibited (no VFA nor ethanol accumulation was recorded), although lactic acid fermentation at such pH is plausible (Itoh et al., 2012; Li et al., 2014). Feng et al. (2011) also monitored $NH_4^+$ and $PO_4^{3-}$ concentrations over time, measurements frequently omitted in publications but utterly important

**Table 2**

Summary of WAS and FW co-fermentation publications studying the mixing ratio and salinity in batch assays.

| Co-substrate (CoS) | T (°C) | pH control | WAS:CoS mixing ratio | $VFA_{max}$ (mgCOD·L$^{-1}$) | Fermentation yield (mg COD·g$^{a-1}$) | Main product | Reference |
|---|---|---|---|---|---|---|---|
| Canteen | 20 | No | 14:86 (%wt) | – | 368 (VSS) | HPro | (Zhao et al., 2016) |
| | | | 14:86 (%wt) + 4 g NaCl/L | – | ~500 (VSS) | HPro | |
| | | | 14:86 (%wt) + 8 g NaCl/L | – | 639 (VSS) | HPro | |
| | | | 14:86 (%wt) + 12 g NaCl/L | – | ~400 (VSS) | HPro | |
| | | | 14:86 (%wt) + 16 g NaCl/L | – | 169 (VSS) | HPro | |
| Potato waste | 35 | 10 | 75:25 (%VS) | – | 152 (VS) | HAc | (Ma et al., 2017) |
| | | | 50:50 (%VS) | – | 268 (VS) | HAc | |
| | | | 25:75 (%VS) | – | 344 (VS) | HAc | |
| Synthetic FW | 35 | 10 | 75:25 (%VS) | – | 140 (VS) | HAc | |
| | | | 50:50 (%VS) | – | 218 (VS) | HAc | |
| | | | 25:75 (%VS) | – | 282 (VS) | HAc | |
| Rice | 35 | No | 90:10 (%wt) | 3114 | – | HAc | (Wu et al., 2020) |
| | | 3 | | 1415 | – | HPro | |
| | | 4 | | 2509 | – | HAc | |
| | | 5 | | 2930 | – | HPro | |
| | | No | 80:20 (%wt) | 4741 | – | HPro | |
| | | 3 | | 1589 | – | HAc | |
| | | 4 | | 4315 | – | HPro | |
| | | 5 | | 4944 | – | HPro | |
| | | No | 70:30 (%wt) | 7671 | – | HPro | |
| | | 3 | | 2235 | – | HAc | |
| | | 4 | | 6012 | – | HPro | |
| | | 5 | | 7738 | – | HPro | |
| OMFSW | 55 | 9 | 20:80 (%V) | 11,124 | 270 (COD) | HAc & HBu | (Cerdán et al., 2021) |
| Artichoke | | | | 10,354 | 360 (COD) | HAc | |
| Apple pulp | | | | 9955 | 350 (COD) | HAc | |
| Microalgae | | | | 7281 | 310 (COD) | HAc | |
| Synthetic FW | 35 | No | 50:50 (%VS) | ~30,000 | 489 (VS) | HBu | (Vidal-Antich et al., 2021) |
| | | | 70:30 (%VS) | ~20,000 | 419 (VS) | HBu | |
| | | | 90:10 (%VS) | ~5000 | 175 (VS) | HPro | |

[a] Fermentation yield denominator unit in brackets.

**Table 3**
Summary of WAS and FW co-fermentation publications in continuous experiments.

| Co-substrate (CoS) | OLR ($g^a \cdot L^{-1} \cdot day^{-1}$) | HRT (days) | T (°C) | pH control | WAS:CoS mixing ratio | VFA$_{max}$ (mgCOD·L$^{-1}$) | Fermentation yield ($mg^b \cdot g^{c^{-1}}$) | Main product | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Rice | – | 8 | 21 | No | 20/1 (C/N) | – | ~150 (COD) (VSS) | HAc | (Feng et al., 2009) |
| | | | | 4 | | – | ~120 (COD) (VSS) | HAc | |
| | | | | 5 | | – | ~280 (COD) (VSS) | HAc | |
| | | | | 6 | | – | ~350 (COD) (VSS) | HPro | |
| | | | | 7 | | – | 406 (COD) (VSS) | HPro | |
| | | | | 8 | | – | 520 (COD) (VSS) | HPro | |
| | | | | 9 | | – | ~480 (COD) (VSS) | HAc | |
| | | | | 10 | | – | ~250 (COD) (VSS) | HAc | |
| | | | | 11 | | – | ~200 (COD) (VSS) | HAc | |
| Synthetic FW | 8 (VSS) | 8 | 35 | 5.5 | 10:90 (%VSS) | 8626 | – | – | (Hong and Haiyun, 2010) |
| | | | | 6.5 | | 25,721 | – | – | |
| | | | | 7.5 | | 24,131 | – | – | |
| | | | | 6.5 | 20:80 (%VSS) | 19,821 | – | – | |
| | 10 (VSS) | 10 | 35 | 6.0 | 05:95 (%VSS) | 3873 | – | – | |
| | | | | 7.0 | | 20,973 | – | – | |
| | | | | 6.0 | 15:85 (%VSS) | 14,309 | – | – | |
| | | | | 7.0 | | 26,541 | – | – | |
| | 8.3 (VSS) | 8.9 | 35 | 6.9 | 12:88 (%VSS) | 29,099 | – | – | |
| Canteen | 8 (VS) | – | 20 | | 17:83 (%VS) | – | 200 (COD) (VS) | – | (Wu et al., 2016) |
| | | | 40 | | | – | 866 (COD) (VS) | – | |
| | | | 60 | | | – | 345 (COD) (VS) | – | |
| | | | 40 | | 25:75 (%VS) | – | 390 (COD) (VS) | – | |
| | | | 40 | | 13:87 (%VS) | – | 590 (COD) (VS) | – | |
| Rice | – | 4 | 25 | 6.5$^d$ | 88:11 (%V) | 3584 | – | HPro | (Li and Li, 2017) |
| Wheat | – | | | | | 4447 | – | HPro | |
| Corn | – | | | | | 4295 | – | HPro & HAc | |
| Bean | – | | | | | 1940 | – | HAc & HPro | |
| Chicken meat | – | | | | | 2572 | – | HAc & HPro | |
| Canteen | 13.7 (VS) | 5 | 55 | 9 | 80:20 (%V) | 14,250 | 288 (VFA) (VS) | HAc | (Garcia-Aguirre et al., 2019) |
| OFMSW | 7.7 (VS) | 6 | 37 | 8–9 | 65:35 (%V) | 39,000 | 740 (COD) (VS) | – | (Moretto et al., 2019) |
| | 9.3 (VS) | 5 | | | | 38,000 | 710 (COD) (VS) | – | |
| | 11.3 (VS) | 4.1 | | | | 31,000 | 600 (COD) (VS) | – | |
| Canteen | – | – | 35 | 9 | 14:86 (%VSS) | 17,200 | – | HPro | (Xu et al., 2020) |

[a] OLR numerator units in brackets.
[b] Fermentation yield numerator units in brackets.
[c] Fermentation yield denominator units in brackets.
[d] The pH was only adjusted at the beginning of the experiment.

when conceiving the potential applications for the fermentation liquor (see discussion in Section 5). Feng et al. (2011) reported a noticeably lower $PO_4^{3-}$ concentration at pH 7–10 than at pH 4–6, which was attributed to the precipitation of calcium and magnesium phosphate salts at higher pH.

Moretto et al. (2019) also evaluated the impact of pH on WAS and FW co-fermentation (65–35% in volume-basis) in batch assays at 37 and 55 °C. Although Moretto et al. (2019) only adjusted the starting pH (pH was not controlled over time), their results aligned with those reported by Feng et al. (2011, 2009) since the highest fermentation yields were achieved at the starting pH of 7 and 9 (final pH at day 10 of ~5.5 and ~6.0, respectively). The pH of the assays starting at pH 5 quickly dropped to pH values of ~4.0, which inhibited the acidogenic fermentation process as illustrated by the lower $COD_{VFA}$/sCOD ratio (the sCOD concentration was relatively constant across the different pH and temperature conditions). Moretto et al. (2019) results showed that pH had a higher influence than temperature on the fermentation yield and product profile. As in Feng et al. (2011), the product profile was dominated by acetic acid, propionic acid and n-butyric acid. Moretto et al. (2019) also operated a continuous stirred-tank reactor (CSTR) WAS-FW co-fermenter at 37 °C where the pH was set between 8 and 9 by adjusting the feedstock pH to 9. The fermenter showed a high fermentation efficiency at an OLR of 7.7 and 9.3 kgVS·m$^{-3}$·day$^{-1}$ (HRT of 6 and 5 days, respectively), but it overloaded (decreased VFA yield) when the OLR was increased to 11.3 kgVS·m$^{-3}$·day$^{-1}$ and the HRT was decreased to 4 days despite the pH remained between 8 and 9.

The results reported by Moretto et al. (2019) and Feng et al. (2011, 2009) are in agreement with those reported by Chen et al. (2013b), who operated five mesophilic (35 °C) semi-continuous fermenters co-fermenting WAS-FW (12% and 88% in VSS-basis) at pH 5, 7, 9, 11 and without pH control. The highest VFA yield was achieved in the fermenters at pH 7 and 9 (no statistical difference), with VFA concentration 2-fold and 10-fold higher than the achieved at pH 11 and pH 5, respectively. The fermenter without pH control showed the worst performance. Chen et al. (2013b) showed a distinct impact of pH on hydrolysis (organic matter solubilisation) and fermentation (soluble organic matter conversion to VFA) since the fermenter at pH 11 showed the highest hydrolysis rate while the fermenter at pH 7 showed the lowest. The distinct impact of pH on hydrolysis and fermentation was also reported by Feng et al. (2011), who reported a higher accumulation of non-VFA soluble COD at pH 10 and 11 when compared to the test at pH 7, 8 and 9. The higher organic matter solubilisation at alkaline pH (pH ≥10), when compared to neutral and acidic pH, is quite consistent across the literature (Garcia-Aguirre et al., 2019; Ma et al., 2019). However, given the high buffer capacity of WAS and the acidic nature of fermentation, large quantities of chemicals would be required to keep the fermenter under alkaline conditions. Besides the increased operating costs and process complexity, high doses of alkaline chemicals could limit the application of biosolids as a fertilizer due to its higher salinity content.

Hong and Haiyun (2010) evaluated how the interaction between different operational variables (i.e., pH, WAS-FW mixing ratio, HRT and OLR) influenced WAS-FW co-fermentation performance by testing 30 different combinations in mesophilic continuous experiments. The highest VFA concentration predicted by the response surface method was WAS-FW 12:88%

(in VSS-basis), HRT of 9 days, OLR of 8 kgVSS·m$^{-3}$·day$^{-1}$ and pH 7. Regarding pH, experimental results showed that pH had a significant interaction with other variables. The optimum pH increased from acidic (~6.0) to neutral (~7.0) values as the OLR increased from 4 to 12 kgVSS·m$^{-3}$·day$^{-1}$ and as the mixture was enriched with FW. These results suggest that a higher pH is needed as the amount of biodegradable organic matter in the system increases, which could be related to the lower product inhibition by undissociated VFA at higher pH (Infantes et al., 2012; Xiao et al., 2016). Hong and Haiyun (2010) also reported a significant interaction between pH and HRT, however, the experimental data did not allow explaining these results. In this regard, Garcia-Aguirre et al. (2019), who operated a pilot-scale WAS-FW co-fermenter (80:20% in volume-basis) at 55 °C, pH 9 and HRT of 5 days, stated that the combination of pH and HRT is a strategy to limit the growth of methanogenic archaea. Nonetheless, the HRT should be long enough to hydrolyse most of the particulate organic matter that can be later fermented into VFA (Garcia-Aguirre et al., 2019).

WAS-FW co-fermentation research has shown that the highest fermentation yields are achieved at circumneutral pH. This is an encouraging outcome since the buffering capacity of WAS can be used to keep the pH around neutrality, hence preventing the inhibition of fermentative bacteria without the need of purchasing chemicals to control the pH. Nonetheless, co-fermentation operational conditions (and pH as a key operational parameter) need to consider conditions where hydrolytic and fermentation activity is high but also conditions where the proliferation and activity of methanogens is limited.

### 2.1.2. Impact of mixing ratio on WAS-FW co-fermentation

The impact of the ratio between WAS and FW on co-fermentation performance has received little attention, although it is one of the most important parameters when designing a WAS-FW co-fermentation process. Table 2 summarises the publications that have studied the impact of WAS-FW mixing ratio on co-fermentation performance. To the best of the authors' knowledge, this factor has only been studied in batch assays. Batch assays are an excellent screening tool. However, the performance of continuous fermenters may differ due to: (i) microbial factors such as the development of a specialised microbial community, the hydraulic selective pressure on the microbial community and microorganisms' constant immigration, (ii) operational parameters such as HRT, OLR and feeding regime, and (iii) process parameters such as pH (in systems without pH control), alkalinity or the concentration of inhibitory compounds.

Ma et al. (2017) evaluated the impact of the WAS-FW mixing ratio at 35 °C and pH 10 using two different wastes, i.e., FW collected from the university canteen and potato peel waste. Specifically, Ma et al. (2017) tested 3 ratios: 75:25%, 50:50% and 25:75% for each FW (VS-basis) as well as three mono-fermentation controls (one for each substrate). The experimental results showed that the VFA yield increased as the amount of FW in the mixture increased. The co-fermentation yields were higher than the theoretical fermentation yields when considering the mono-fermentation yield and the proportion of each waste in the mixture. An increase of co-fermentation yields with an increasing proportion of FW was also reported by Wu et al. (2020) and Vidal-Antich et al. (2021). Vidal-Antich et al. (2021) co-fermented WAS and synthetic FW (90:10%, 70:30% and 50:50% in VS-basis) at 35 °C. In contrast to Ma et al. (2017), Vidal-Antich et al. (2021) did not control the pH and relied on the WAS alkalinity to keep the pH above inhibitory levels. Both publications showed that the higher biodegradability of FW is the major contributor to the fermentation yield. Vidal-Antich et al. (2021) results also indicated that WAS alkalinity is an important parameter for systems without pH control since the maximum fermentation yield from FW (~900 mgCOD·gVS$^{-1}$) was achieved in co-fermentation experiments at pH above 5.0. The poor performance of WAS-FW co-fermentation at pH below 5.0 was also observed by Wu et al. (2020) who co-fermented three WAS-FW mixtures (90:10%, 80:20% and 70:30% in TS-basis) at 35 °C at four different pH (i.e., 3, 4, 5 and without pH control).

Regarding the product profile, Ma et al. (2017) reported that acetic acid was the main fermentation product in both mono- and co-fermentation

tests (40–60% in COD-basis), which aligns with fermentation experiments carried out at alkaline conditions (pH 9–11) (Cerdán et al., 2021; Cheah et al., 2019; Garcia-Aguirre et al., 2019). The authors reported that co-fermentation mixtures with potato peel waste (starch-rich waste) favoured the accumulation of butyric acid and ethanol, while co-fermentation mixtures with canteen waste favoured the accumulation of propionic acid and valeric acid. On the other hand, Vidal-Antich et al. (2021) reported that butyric acid accumulation was favoured, to the detriment of propionic acid, as the proportion of FW in the mixture increased and the pH decreased. Interestingly, both studies observed a consumption of acetic acid during the last days of the fermentation batch, particularly in the mixtures with a higher proportion of WAS. As discussed by Vidal-Antich et al. (2021), mixtures with a high proportion of WAS may not be favourable for WAS-FW co-fermentation, especially when acetic acid is the desired fermentation product. In batch experiments, the consumption of acetic acid could be related to the proliferation of acetic acid consuming microbes (e.g., methanogenic archaea) (Jia et al., 2013). Additionally, in continuous fermenters, the constant immigration of microorganisms with WAS (e.g., phosphorous accumulating organisms) may also prevent acetic acid accumulation (Wu et al., 2016). Microorganisms' immigration also increases the stochasticity of the fermenters' microbial community assembly (Vrieze et al., 2020; Yuan et al., 2019), however, the impact of immigration on co-fermentation performance is still unknown.

### 2.1.3. Impact of FW composition on WAS-FW co-fermentation

FW is a heterogeneous waste which seasonal composition depends on multiple factors (e.g., geographic, economic, political, climatologic). Accordingly, some authors have evaluated the impact of FW composition on WAS-FW co-fermentation performance. Also, the addition of FW and food processing waste (e.g., fish processing residues) can increase the salinity of the fermentation liquor.

Li and Li (2017) evaluated in batch experiments (25 °C and without pH control) the impact of different FW constituents (i.e., rice, wheat, corn, bean and chicken) on WAS-FW co-fermentation performance. Rice, wheat and corn are carbohydrate-rich substrates, chicken meat is a protein-rich substrate, while beans have both carbohydrates and protein. Experimental results showed that the carbohydrate-rich substrates reached higher fermentation yields (wheat > corn > rice) followed by chicken and beans. The lower fermentation yield of chicken and beans was related to hydrolysis limitations. These results illustrate that both macromolecules composition (carbohydrates, protein and lipids) and bioavailability (macromolecule structure) are important factors to assess the fermentability of a substrate. Li and Li (2017) also reported a different VFA profile between (i) rice, wheat and corn, and (ii) chicken and beans; however, these two groups had also a different pH (the final pH was 4.6 and 5.5, respectively). The VFA profile of the carbohydrate-based substrates (rice, wheat and corn) was dominated by propionic acid and acetic acid but also enriched in butyric acid and valeric acid. On the other hand, the VFA profile of beans and chicken was mainly acetic acid and propionic acid.

The product profiles reported by Li and Li (2017) were quite different from those reported by Ma et al. (2017), who co-fermented WAS-FW at pH 10 using two different waste (canteen FW and potato peel waste). In Ma et al. (2017), acetic acid was the main VFA for both substrates and mixtures; however, potato peel waste (carbohydrate-rich substrate) favoured the accumulation of ethanol and butyric acid, while canteen waste favoured the accumulation of propionic acid and valeric acid. Ma et al. (2017) attributed these product profile differences to the carbohydrate, protein and lipid proportion of the mixture. These conclusions were further supported by co-fermentation experiments using glucose (carbohydrate model substrate) and bovine serum albumin (BSA, protein model substrate). The former favoured the accumulation of acetic acid and butyric acid, while the latter favoured the accumulation of acetic acid and valeric acid. Bevilacqua et al. (2021, 2020) reported that protein composition (casein vs. gelatine) had an impact on fermentation yield and product profile, which illustrates the complexity of predicting the fermentation yield and product profile of

waste streams. Little attention has been given to the role of lipids on co-fermentation performance. Peces et al. (2020) co-fermented three mixtures between WAS and oleic acid (lipid model substrate) at 20 °C, without pH control and under semi-aerobic conditions. The VFA production increased as the amount of oleic acid in the mixture increased, however, the fermentation extent of oleic acid was rather low (~5%). The addition of oleic acid promoted the accumulation of butyric and caproic acid when compared to the WAS mono-fermentation control.

Zhao et al. (2016) studied the impact of salinity on WAS-FW (15:85% in weight-basis) co-fermentation at 20 °C by adding different NaCl concentrations (i.e., 0, 4, 8, 12, and 16 g·L$^{-1}$). Co-fermentation results showed that a higher degree of hydrolysis was achieved as the NaCl concentration increased. However, the maximum VFA yield was obtained at 8 g·L$^{-1}$ indicating that high salinity values can inhibit the fermentation of the hydrolysed compounds. The positive impact of salinity on hydrolysis and the negative impact of salinity on fermentation and methanogenesis were further supported by experiments carried out using model substrates (i.e., BSA, dextran, alanine, glucose and acetic acid). Zhao et al. (2016) results imply that the salinity of the fermentation liquor can have an impact on fermentation results, which is in agreement with other publications (He et al., 2019; Jin et al., 2016; Li et al., 2021). However, in a continuous fermenter, the microbial community could adapt over time to tolerate high salinity (halotolerant microbial community), which could increase the inhibition tolerance to saline environments and lead to higher fermentation yields and different products profile.

### 2.1.4. Impact of temperature on WAS-FW co-fermentation

Temperature is an important operational factor on (co-)fermentation because it affects (i) biochemical reactions (e.g., metabolic rates, enzymatic activity, microorganisms growth and decay rates), (ii) physico-chemical processes (e.g., mass transfer, gas solubility, chemical equilibrium), and (iii) microbial community assembly (Esteban-Gutiérrez et al., 2018; Fernández-Domínguez et al., 2020; Jiang et al., 2021; Strazzera et al., 2018). Despite its importance, the role of temperature on WAS-FW co-fermentation has only been studied as a covariate in batch assays. Batch assays results are strongly driven by the capabilities of the starting microbial community, therefore, microbial acclimation to different operational conditions and substrate composition could lead to different responses under continuous conditions.

Chen et al. (2013a) analysed the impact of several factors on WAS-FW co-fermentation (i.e., pH, C/N ratio, temperature) using the response surface methodology. Chen et al. (2013a) reached the highest fermentation yield at 35 °C, pH 8 and C/N ratio 22 (WAS-FW mixture of 40:60 in TS-basis). Mesophilic conditions were also the temperature regime that showed the highest fermentation yield in Moretto et al. (2019) and Li et al. (2014). Li et al. (2014), who co-fermented WAS-FW (20:80% in TS-basis) at four different temperatures (20, 35, 50 and 65 °C), reported that 20 and 35 °C favoured acidogenic fermentation (mainly acetic acid and propionic acid), while 50 and 65 °C conditions favoured lactic fermentation. Li et al. (2014) did not report the pH of the fermentation liquor, although pH is well-known to drive the fermentation product profile. In fact, Moretto et al. (2019), who co-fermented WAS-FW under different pH and temperatures (35 and 55 °C), reported that pH had a higher influence than temperature on the fermentation yield and product profile. Moretto et al. (2019) reported the highest fermentation yield at mesophilic conditions and a starting pH of 7 and 9 (no statistical difference). Moretto et al. (2019) also showed that thermophilic conditions result in higher organic matter solubilisation than mesophilic conditions, in agreement with other publications (Capson-Tojo et al., 2018; Fernández-Domínguez et al., 2020; Garcia-Aguirre et al., 2017; Hasan et al., 2015).

### 2.2. WAS co-fermentation with agro-industrial waste

Agro-industrial waste (AgriW) has mainly been co-fermented with WAS to (i) balance the C/N ratio and (ii) increase the amount of biodegradable waste in the fermentation liquor. On the other hand, WAS provides the moisture, buffer capacity and microbial community lacking in most AgriW. AgriW is more homogeneous than FW, but it has a seasonal availability. The AgriW evaluated in the literature comprise a variety of residues, including perennial ryegrass (Jia et al., 2013), corn and rice straw (Guo et al., 2015; Xin et al., 2018), spent mushroom substrate (Guo et al., 2015), henna leaves powder (Huang et al., 2016), tofu residues (Huang et al., 2019) and soy sauce residues (Duan et al., 2019). Table 4 summarises the WAS-AgriW co-fermentation publications. AgriW, especially plants residues, are lignocellulosic substrates where the biodegradable carbon (hemicellulose and cellulose) is trapped in the lignocellulosic matrix making it unavailable for biodegradation (Abdelaziz et al., 2016; Duan et al., 2019; Jia et al., 2013). Accordingly, most publications co-fermenting WAS and lignocellulosic AgriW have worked with pre-treated AgriW (Duan et al., 2019; Guo et al., 2015; Huang et al., 2016; Xin et al., 2018). However, pre-treatments require an economic evaluation because revenues from higher fermentation yields need to compensate the pre-treatment capital and operating costs (e.g., chemicals and/or energy) (Fang et al., 2019; Huang et al., 2016).

Jia et al. (2013) evaluated in batch assays (35 °C, without pH control) the impact of the C/N ratio (from 7/1 to 26/1) on WAS and perennial ryegrass co-fermentation. Experimental results showed that mixtures with a C/N ratio of 18/1, 20/1 and 22/1 reached the highest fermentation yield, which falls within the optimum C/N ratio range reported in the literature (Fang et al., 2020, 2019; Huang et al., 2019; Rughoonundun et al., 2012; Xia et al., 2016). The assays carried out at the optimum C/N ratio showed a higher degradation extent of carbohydrates (hemicellulose, cellulose and lignin) and protein that was attributed to a higher enzymatic activity due to the balanced C/N ratio. The tests at the optimum C/N ratio (i.e., 18/1, 20/1 and 22/1) had the lowest pH of all tests (pH of ~4.8), which may have limited the methanogenic activity and favoured the accumulation of acetic acid in the fermentation liquor. Xin et al. (2018) co-fermented in mesophilic batch assays (i) pre-treated WAS with corn straw and (ii) pre-treated WAS with corn straw and pig manure at a C/N ratio of 20. Experimental results showed that co-fermentation is a suitable strategy to increase fermentation yields and that carbohydrate-based co-substrates promote the accumulation of acetic acid, which aligned with the results reported by Jia et al. (2013) and Huang et al. (2019).

An increased fermentation yield and a shift of the product profile under co-fermentation conditions were also reported by Huang et al. (2016) and Huang et al. (2019), who screened in mesophilic batch assays several mixtures between WAS and henna leaves, and WAS and tofu residue, respectively. Huang et al. (2016) achieved the highest VFA concentration in the henna leave mono-fermentation test, which was related to the presence of easily hydrolysable organic matter, the low pH of the test (6.0–6.5) and the higher concentration of lawsone (henna plant secondary metabolite) in the fermentation liquor. The positive impact of lawsone on fermentation was related to its capability to (i) act as an electron mediator and (ii) inhibit methanogenic archaea. The role of crops and plants secondary metabolites (e.g., terpenes, steroids, phenols, alkaloids, flavonoids) on waste fermentation warrants further research since some of them have antimicrobial or antioxidant properties that could inhibit bacterial and archaeal activity (Erb and Kliebenstein, 2020; Pang et al., 2021). However, in Huang et al. (2016), the low concentration of soluble carbohydrates and soluble protein at the end of the mono-fermentation test suggests that the fermentative bacteria were not inhibited. In fact, the henna leave mono-fermentation control was the only assay where acetic acid was not consumed at the end of the test suggesting that methanogenic archaea were inhibited by lawsone. Huang et al. (2019) evaluated the impact of isoflavones (from 0 to 20 mg·L$^{-1}$) on WAS and tofu residues co-fermentation and concluded that the presence of isoflavones in the fermentation liquor did not affect organic matter solubilisation. However, the impact on fermentation yield and fermentation profile was not reported.

To the best of the authors' knowledge, Guo et al. (2015) is the only study that has evaluated the co-fermentation of WAS and AgriW under continuous conditions. Two different types of substrates were evaluated: straw

**Table 4**

Summary of WAS and AgriW co-fermentation publications.

| Co-substrate (CoS) | Reactor type | SRT (days) | T (°C) | pH control | WAS:CoS mixing ratio | VFA$_{max}$ (mg COD·L$^{-1}$) | Fermentation yield (mg COD·g$^{a-1}$) | Main product | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Perennial ryegrass | Batch | – | 35 | No | 24/1 (C/N) | – | 230 (TS) | HAc | (Jia et al., 2013) |
| | | | | | 22/1 (C/N) | – | 320 (TS) | HAc | |
| | | | | | 20/1 (C/N) | – | 369 (TS) | HAc | |
| | | | | | 18/1 (C/N) | – | 350 (TS) | HAc | |
| | | | | | 16/1 (C/N) | – | 220 (TS) | HPro | |
| | | | | | 14/1 (C/N) | – | 108 (TS) | HPro | |
| | | | | | 12/1 (C/N) | – | 97 (TS) | HPro | |
| | | | | | 10/1 (C/N) | – | 60 (TS) | HPro | |
| | | | | | 8/1 (C/N) | – | 42 (TS) | HPro | |
| Corn stalk | CSTR | 10 | 35 | 10 | 66:33 (%VSS:VS) | 8743 | 416 (VS) | HAc | (Guo et al., 2015) |
| Rice straw | | | | | | 9044 | 431 (VS) | HAc | |
| *Lentinula edodes* | | | | | | 5576 | 265 (VS) | HAc | |
| *Agaricus bisporus* | | | | | | 5880 | 280 (VS) | HAc | |
| Corn stalk | | 8 | 35 | 10 | | 9943 | 473 (VS) | HAc | |
| Rice straw | | | | | | 10,492 | 500 (VS) | HAc | |
| *Lentinula edodes* | | | | | | 6272 | 299 (VS) | HAc | |
| *Agaricus bisporus* | | | | | | 6602 | 314 (VS) | HAc | |
| Corn stalk | | 5 | 35 | 10 | | 9039 | 430 (VS) | HAc | |
| Rice straw | | | | | | 9247 | 440 (VS) | HAc | |
| *Lentinula edodes* | | | | | | 4859 | 231 (VS) | HAc | |
| *Agaricus bisporus* | | | | | | 4899 | 233 (VS) | HAc | |
| Henna leaves | Batch | – | 35 | 8[b] | 25:75 (%TS) | 7875 | – | HAc & HPro | (Huang et al., 2016) |
| | | | | | 50:50 (%TS) | 5326 | – | HAc & HPro | |
| | | | | | 75:25 (%TS) | 3910 | – | HAc & HPro | |
| Corn stalk | Batch | – | 35 | No | 1:25 (gCoS·L$_{WAS}^{-1}$) | 4800 | – | HAc | (Xin et al., 2018) |
| | | | | | 1:31 (gCoS:L$_{WAS}^{-1}$) | 5100 | – | HAc & HPro | |
| Soy sauce residue (AH) | Batch | – | 35 | No | 66:33 (%VSS:VS) | 5300 | – | HAc | (Duan et al., 2019) |
| Soy sauce residue (SA) | | | | | | 3350 | – | HAc | |
| Soy sauce residue (TA) | | | | | | 4517 | – | HAc | |
| Tofu residue | Batch | – | 37 | No | 14:86 (%VSS) | – | 75 (VSS) | HAc & HPro | (Huang et al., 2019) |
| | | | | | 24:76 (%VSS) | – | ~120 (VSS) | HAc & HPro | |
| | | | | | 32:68 (%VSS) | – | ~175 (VSS) | HAc | |
| | | | | | 39:61 (%VSS) | 4716 | 241 (VSS) | HAc | |
| | | | | | 49:51 (%VSS) | – | ~175 (VSS) | HBu | |
| Winery wastewater | CSTR | – | 55 | 9 | 50:50 (%V) | 19,100 | 490 (COD) | HAc | (Esteban-Gutiérrez et al., 2018) |
| Meat and bone meal | | | | | 90:10 (%V) | 23,200 | 310 (COD) | HAc | |

AH: ammonium hydroxide pre-treated.

TA: thermo-alkaline pre-treated.

SA: sulphuric acid pre-treated.

[a] Fermentation yield denominator unit in brackets.

[b] The pH was only adjusted at the beginning of the experiment.

residues (i.e., corn and rice straw) and spent mushroom substrate from two different mushroom crops (i.e., *Lentinula edodes* and *Agaricus bisporus*). The AgriW were pre-treated using an alkaline solution (2% NaOH, 85 °C, 1 h). To prepare the fermenter's feedstock, the pre-treated AgriW was mixed with WAS at a 1:2 ratio (VS$_{AgriW}$:VSS$_{WAS}$) and the pH was adjusted to 10. The five fermenters (including one WAS mono-fermentation control) were operated at 35 °C and three different HRT (10, 8 and 5 days), with the OLR increasing as the HRT decreased. Experimental results showed that co-fermentation with corn straw and rice straw resulted in higher VFA yields than co-fermentation with the spent mushroom substrates (almost a two-fold increase). The co-fermenters achieved the maximum VFA concentration and VFA yield when operated at an HRT of 8 days; however, the concentration of soluble carbohydrates in the fermentation liquor decreased as the HRT decreased. These results indicate that, despite their pre-treatment, co-substrates fermentation was limited by hydrolysis. Finally, the microbial community analysis carried out at the end of the fermenter's operation showed that the microbial community was dominated by the phyla *Firmicutes*, *Bacteroidetes* and *Proteobacteria* and that substrate type clustered the microbial community of the fermenters. Jia et al. (2013), Xin et al. (2018) and Duan et al. (2019) also analysed the microbial community of WAS-AgriW co-fermentation. However, the microbial community results of batch experiments will not be discussed in this review

as their results and implications are considered dubious by the authors (see further discussion in Section 6).

## 3. Primary sludge co-fermentation

Some WWTPs have already implemented PS fermenters to produce the extra soluble organic matter needed for biological nutrient removal, particularly WWTPs with enhanced biological phosphorus removal (EBPR) (Metcalf & Eddy et al., 2002; Oldham et al., 1994). PS is more easily biodegradable than WAS, therefore, it provides higher mono-fermentation yields (Feng et al., 2011; Ji et al., 2010). The co-fermentation of PS with other organic waste has received less attention than co-fermentation with WAS. This may be related to (i) its heterogeneity and variable composition, which may hinder controlling the fermentation yield and profile, (ii) its lower buffer capacity, which limits the amount of co-substrate added to keep the pH above inhibitory levels, and (iii) the lower biogas production in the anaerobic digester if PS is diverted to VFA production. However, due to the limited research, the benefits and constraints of PS co-fermentation remain unclear. Table 5 summarises the PS co-fermentation publications.

Banerjee et al. (1999) pioneered PS co-fermentation research with a study devoted to the co-fermentation of PS and potato-processing wastewater

(starch-rich WW) under continuous conditions. Specifically, Banerjee et al. (1999) co-fermented PS and potato WW (50:50% in volume-basis) under different HRT (18 and 30 h) and temperature (22, 30 and 35 °C) at an SRT of 7 days. Experimental results showed that adding potato WW as a co-substrate increased by 39% the VFA concentration. Despite the lower OLR, the VFA concentration was further increased (37%) when the HRT was increased from 18 to 30 h. Considering the particulate nature of both wastes, the latter results suggest that the efficiency of the fermentation process was limited by hydrolysis, although the results could also be explained by a more favourable pH (unfortunately, the pH was not reported). Regarding the impact of temperature, Banerjee et al. (1999) reported that the optimum temperature for the co-fermenter was 30 °C while the optimum temperature for the PS mono-fermenter control was 22 °C (room temperature). Both fermenters showed the worse performance at 35 °C, however, the experimental results did not allow elucidating the causes of such results. Temperatures around 35 °C have been frequently acknowledged as the optimum temperature for fermentation (Ramos-Suarez et al., 2021; Strazzera et al., 2018).

Min et al. (2005) co-fermented PS and FW (90:10% and 75:25% in weight-basis) under different HRT (1, 3 and 5 days), temperature (18 and 35 °C), and without and with pH control (pH of ~6.5). Min et al. (2005)

also reported higher co-fermentation yields at room temperature (18 °C) than at 35 °C. Experimental results showed that mesophilic conditions, circumneutral pH and long HRTs favoured biogas production and prevented the accumulation of VFA. Experimental results were not conclusive about the impact of the mixture composition, but in general higher fermentation yields were achieved from the 90:10% PS-FW mixture when compared to the 75:25% PS-FW mixture. Thus, providing more co-substrate or external alkalinity to adjust the pH from ~5.5 to ~6.5 did not improve the fermenters' efficiency. Min et al. (2005) results reinforce the idea that fermenters' operational conditions should apply enough selective pressure to limit the proliferation and activity of methanogenic archaea. To limit the presence of methanogenic archaea in PS, the anaerobic digester supernatant should be returned to the water line after the primary settler.

Owusu-Agyeman et al. (2021) studied in batch assays the co-fermentation of PS and FW (30:70% in volume-basis) at 35 °C and two distinct starting pH (5 and 10). However, their study focused on the impact of direct enzymes addition and pre-treatments (enzymatic, ultrasonic, thermic) on co-fermentation performance. Co-fermentation controls (without enzyme addition or pre-treatment) showed that tests starting at pH 10

**Table 5**
Summary of co-fermentation publications using PS as a main substrate.

| Co-substrate (CoS) | Reactor type | HRT (days) | T (°C) | pH control | PS:CoS mixing ratio | VFA$_{max}$ (mg$^a$·L$^{-1}$) | Fermentation yield (mg COD·g VS$^{-1}$) | Main product | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Potato WW | CSTR | 0.75 | 22 | No | 50:50 (%V) | 514 (COD) | – | HAc | (Banerjee et al., 1999) |
| | | 1.25 | | | | 627 (COD) | – | HAc | |
| | | | 30 | | | 713 (COD) | – | HAc | |
| | | | 35 | | | 419 (COD) | – | HAc | |
| FW | CSTR | 1 | 18 | Yes[b] | 90:10 (%wt) | 150 (VFA) | – | HBu | (Min et al., 2005) |
| | | | | No | | 270 (VFA) | – | HBu | |
| | | | | Yes[b] | 75:25 (%wt) | 2155 (VFA) | – | HPro | |
| | | | | No | | 3610 (VFA) | – | HPro & HBu | |
| | | 3 | | Yes[b] | 90:10 (%wt) | 330 (VFA) | – | HPro & HBu | |
| | | | | No | | 1190 (VFA) | – | HAc | |
| | | | | Yes[b] | 75:25 (%wt) | 450 (VFA) | – | HPro | |
| | | | | No | | 1000 (VFA) | – | HPro & HBu | |
| | | 5 | | Yes[b] | 90:10 (%wt) | 350 (VFA) | – | HBu | |
| | | | | No | | 1185 (VFA) | – | HPro & HBu | |
| | | | | Yes[b] | 75:25 (%wt) | 55 (VFA) | – | HPro & HBu | |
| | | | | No | | 255 (VFA) | – | HPro & HBu | |
| | | 1 | 35 | Yes[b] | 90:10 (%wt) | 255 (VFA) | – | HBu | |
| | | | | No | | 680 (VFA) | – | HPro | |
| | | | | Yes[b] | 75:25 (%wt) | 515 (VFA) | – | HBu | |
| | | | | No | | 2520 (VFA) | – | HPro & HBu | |
| | | 3 | | Yes[b] | 90:10 (%wt) | 350 (VFA) | – | HPro & HBu | |
| | | | | No | | 405 (VFA) | – | HPro & HBu | |
| | | | | Yes[b] | 75:25 (%wt) | 220 (VFA) | – | HBu | |
| | | | | No | | 360 (VFA) | – | HPro | |
| | | 5 | | Yes[b] | 90:10 (%wt) | 280 (VFA) | – | HBu | |
| | | | | No | | 310 (VFA) | – | HBu | |
| | | | | Yes[b] | 75:25 (%wt) | 35 (VFA) | – | HBu | |
| | | | | No | | 160 (VFA) | – | HBu | |
| FOG | CSTR | – | 20–27 | No | 69:31 (%COD) | 3250 (COD) | – | – | (Nicholson et al., 2013) |
| FOG | CSTR | – | 19–25 | No | 80:20 (%COD) | ~800 (COD) | – | – | (Long et al., 2014) |
| | | | 26–30 | | | ~1000 (COD) | – | – | |
| | | | 26–30 | | 89:11 (%COD) | ~600 (COD) | – | – | |
| | | | 20–23 | | 97:3 (%COD) | ~400 (COD) | – | – | |
| | | | 17–20 | | 99:1 (%COD) | ~400 (COD) | – | – | |
| | | | 11–18 | | 96:4 (%COD) | ~400 (COD) | – | – | |
| | | | 20 | | 89:11 (%COD) | ~900 (COD) | – | – | |
| | | | 22–24 | | 82:18 (%COD) | ~600 (COD) | – | – | |
| Oleic acid | Batch | | 20 | No | 66:33(%VS) | 2680 (COD) | 41 | HAc | (Peces et al., 2020) |
| | | | | | 50:50 (%VS) | 4516 (COD) | 46 | HAc & HPro | |
| | | | | | 33:66 (%VS) | 4155 (COD) | 30 | HAc & HPro | |
| FW | Batch | | 35 | 10[d] | 70:30 (%V) | 11,875 (COD) | 486 | HAc | (Owusu-Agyeman et al., 2021) |
| FW[c] | | | | | | 15,323 (COD) | 633 | HAc | |
| FW | | | | 5[d] | | 8008 (COD) | 307 | HPro | |
| FW[c] | | | | | | 6002 (COD) | 215 | HPro | |

[a] VFA$_{max}$ concentration numerator unit in brackets.
[b] The pH was only adjusted between 6.2 and 6.7.
[c] FW was pre-treated with enzymes.
[d] The pH was only adjusted at the beginning of the experiment.

(final pH ~6.0) resulted in higher VFA yields than the tests starting at pH 5 (final pH ~5.0), which was attributed to the higher hydrolytic and fermentative activity under alkaline conditions. The direct enzyme addition also showed better performance in tests starting at pH 10 (increasing the fermentation yield by 30%), whereas the direct enzyme addition at pH 5 did not show any improvement. Pre-treatments improved the fermentation kinetics (the maximum fermentation yield was reached earlier) but they did not improve the fermentation yields. Owusu-Agyeman et al. (2021) acknowledged that the improvements in process performance were not sufficient to justify the pre-treatments costs.

Nicholson et al. (2013), Long et al. (2014) and Peces et al. (2020) studied the co-fermentation of PS and fat, oil and grease (FOG), which can be obtained from the WWTP aerated grit chamber, restaurants grease traps and industrial dissolved air flotation units. FOG is a potential co-substrate due to its high COD density, high biodegradability and low nutrient content. However, the improvements achieved in continuous and batch experiments were lower than expected since the FOG fermentation extent was below 10%. The poor performance of PS-FOG co-fermentation has been related to several factors: (i) FOG low solubility in water, particularly in its unsaturated form (e.g., stearic acid (18:0), palmitic acid (16:0) and myristic acid (14:0)), (ii) its slow biodegradability, and (iii) its capacity to inhibit fermentative bacteria (Long et al., 2014; Nicholson et al., 2013; Varin et al., 2016). Peces et al. (2020), who co-fermented FOG with PS or WAS, achieved a higher extent of FOG fermentation when FOG was co-fermented with WAS, which was attributed to the sludge composition, the sludge microbial community and/or an improved nutrient balance. Although Peces et al. (2020) did not elucidate the mechanisms behind such differences, the difference in fermentation yield and product profile between PS-FOG and WAS-FOG co-fermentation suggests that the inherent properties of the sludge have an important role in co-fermentation performance.

Nicholson et al. (2013) and Long et al. (2014) investigated PS-FOG co-fermentation in a pilot-plant installed in a WWTP. The pilot-plant was operated at several SRT (3, 5 and 7 days), at ambient temperature (10–25 °C), without pH control, and at an $OLR_{PS}$ of ~10 kgCOD·m$^{-3}$·day$^{-1}$. Nicholson et al. (2013) experimental results at an SRT of 5 days showed that increasing the fermenter OLR by 20% using FOG slightly increased the sCOD production from 0.75 to 0.80 kgCOD·m$^{-3}$·day$^{-1}$, where VFA represented 60–75% and 60–85% of the sCOD, respectively. Long et al. (2014) reached the highest co-fermentation yields (~1.0 kgCOD·m$^{-3}$·day$^{-1}$) when the fermenter was operated at an SRT of 3 days. This SRT was used by Long et al. (2014) to pre-design the fermentation facility in the WWTP and conduct an economic analysis. The construction cost of the fermentation facility was estimated at 3–4 million USD, while the VFA produced from PS-FOG co-fermentation could save up to 400,000 USD per year in methanol purchasing costs. Long et al. (2014) results also showed that the fermentation liquor has a relatively high nutrient concentration (N and P) and their preliminary model predicted an increase of total phosphorous concentration in the activated sludge effluent. Finally, it is worth mentioning that Frigon et al. (2006) found that some foam-forming microorganisms in the activated sludge system had a competitive advantage when growing on lipids. Accordingly, the presence of unfermented lipids in the fermentation liquor should be minimised to prevent the occurrence of foaming events in the activated sludge system.

## 4. Sewage sludge co-fermentation

Sewage sludge (SS) refers to the mixture of PS and WAS. SS properties are expected to be more variable across studies than PS and WAS, since its properties depend on (i) the composition of both PS and WAS, (ii) the proportion between PS and WAS (typically ranging between 70:30% and 50:50% in weight-basis), (iii) seasonal dynamics (e.g., wastewater flow rate, composition and temperature), and (iv) the configuration and operation of the WWTP (Astals et al., 2013; Palatsi et al., 2021; Smith et al., 2009). Most publications using SS have provided limited information about the SS origin and composition, which hinders the understanding

and comparison of the experimental results on co-fermentation performance. Table 6 summarises the SS co-fermentation publications.

Li et al. (2013) and Fernando-Foncillas and Varrone (2021) co-fermented SS and FW with two distinct goals. Li et al. (2013) evaluated which operational conditions (i.e., pH, SS-FW mixing ratio and temperature) favoured the accumulation of propionic acid, while Fernando-Foncillas and Varrone (2021) evaluated which operational conditions (i.e., pH, SS-FW mixing ratio and food-to-microorganism ratio) favoured the accumulation of caproic acid. Li et al. (2013) achieved the maximum propionic acid concentration at pH 8 and 9 (SS-FW 15:85% VS-basis, 20 °C), with propionic acid concentration dropping outside these pH values (especially at pH 10). The highest propionic acid concentration was achieved at 20 °C followed by 50 °C and 35 °C (SS-FW 15:85% VS-basis, pH 8). Regarding the influence of the mixture composition (pH 8, 20 °C), mixtures with a higher proportion of SS (i.e., SS-FW 60:40%, 30:70% and 15:85% in VS-basis) led to higher propionic acid concentrations than the 90:10% and 95:5% mixtures. The higher propionic acid concentration in the mixtures with more SS could be related to the protein content of SS (Luo et al., 2021; Ma et al., 2017). Fernando-Foncillas and Varrone (2021) co-fermented SS-FW in batch and continuous assays at 37 °C. Batch assays (response surface methodology) showed that VFA and caproic acid concentrations increased as the proportion of FW in the mixture and the food-to-microorganism ratio increased. Regarding pH, the highest VFA concentration was achieved at pH 9 while the accumulation of caproic acid was favoured at neutral pH. The highest caproic acid concentration at neutral pH was attributed to the occurrence of chain elongation, which could have used the ethanol (7 g·L$^{-1}$) or lactic acid (13 g·L$^{-1}$) in the pre-treated FW as electron donor. The continuous fermenter was fed with two SS-FW mixtures 70:30% and 20:80% (VS-basis) at pH 9, where each mixture was operated at 4-day and 2-day HRT. Additionally, the 70:30% mixture was operated at 6-day HRT. For the 70:30% mixture, operating the fermenter at a 6-day HRT did not prevent the proliferation of methanogens as shown by the noticeable methane production and limited accumulation of VFA (<1.5 gCOD·L$^{-1}$). Decreasing the HRT to 4 days reduced methane production and promoted the accumulation of VFA (up to 4 gCOD·L$^{-1}$), particularly propionic acid. Further decreasing the HRT to 2 days decreased the VFA concentration (~3 gCOD·L$^{-1}$), indicating that the system was overloaded (the OLR of this experiment was not reported). The caproic acid and the acetic acid concentration for the 70:30% mixture at the three HRT was negligible; the latter could explain the observed methane production. Higher VFA yields (8–10 gCOD·L$^{-1}$) were reached when co-fermenting the 20:80% mixture at 4-day and 2-day HRT (OLR of 15 and 30 kgVS·m$^{-3}$·day$^{-1}$, respectively). Similar to the 70:30% mixture, the VFA concentration did not increase when the HRT was reduced to 2-days, despite the higher OLR. At 2-day HRT, caproic acid was the dominant VFA. Based on the consumption of lactic acid, the acidic pH (~5.5) and the low concentration of propionic acid, the authors proposed that chain elongation took place using lactic acid as an electron donor.

Rughoonundun et al. (2012) and Fang et al. (2019) co-fermented SS and lignocellulosic AgriW (pre-treated bagasse and spent mushroom substrate, respectively), screening mixtures between 0:100% and 100:0%, to assess the impact of C/N ratio on batch co-fermentation performance (see Table 6). Although Rughoonundun et al. (2012) conducted their experiments at 55 °C and Fang et al. (2019) at 30 °C, both studies reached similar outcomes: (i) the mono-fermentation yield from sewage sludge was higher than the AgriW, (ii) acetic acid was the main VFA in the fermentation liquor regardless of the substrate composition (both studies added chemicals to inhibit methanogenesis), and (iii) the C/N ratio could not be used as a single factor to explain the improved performance of co-fermentation. Similar conclusions were reported by Luo et al. (2021) who co-fermented SS and paper waste (25:75%, 50:50% and 75:25% in volume-basis) at 35 °C and at three pH conditions (i.e., 5, 10 and without pH control). Rughoonundun et al. (2012) observed that the co-fermentation yield was constant for SS-AgriW mixtures between 30:70% and 80:20% (in TS-basis) where the C/N ratio varied from 24.5 to 9.3, respectively. Fang et al. (2019) reported the highest VFA yield for the 50:50% mixture in

**Table 6**
Summary of co-fermentation publications using SS as a main substrate.

| Co-substrate (CoS) | Reactor type | HRT (days) | T (°C) | pH control | SS:CoS mixing ratio | VFA$_{max}$ (mg$^a$·L$^{-1}$) | Fermentation yield (mg$^b$·g$^{c-1}$) | Main product | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Bagasse[d] | Batch | – | 55 | 7 | 20:80 (%TS) | 13,360 (VFA) | 300 (VFA) (VS) | HAc | (Rughoonundun et al., 2012) |
| | | | | | 30:70 (%TS) | 15,110 (VFA) | 360 (VFA) (VS) | HAc | |
| | | | | | 40:60 (%TS) | 15,080 (VFA) | 360 (VFA) (VS) | HAc | |
| | | | | | 60:40 (%TS) | 14,230 (VFA) | 360 (VFA) (VS) | HAc | |
| | | | | | 80:20 (%TS) | 13,300 (VFA) | 350 (VFA) (VS) | HAc | |
| FW | Batch | – | 20 | No | 14:86 (%VS) | ~3000 (COD$_{HPro}$) | – | HPro | (Li et al., 2013) |
| | | | | 6 | | ~4000 (COD$_{HPro}$) | – | HPro | |
| | | | | 7 | | ~5000 (COD$_{HPro}$) | – | HPro | |
| | | | | 8 | | 7120 (COD$_{HPro}$) | – | HPro | |
| | | | | 9 | | ~6000 (COD$_{HPro}$) | – | HPro | |
| | | | | 10 | | 1020 (COD$_{HPro}$) | – | HPro | |
| | CSTR | – | 20 | 8 | 59:41 (%VS) | 6110 (COD$_{HPro}$) | – | HPro | |
| | | | | | 29:71 (%VS) | 6390 (COD$_{HPro}$) | – | HPro | |
| | | | | | 10:90 (%VS) | 7120 (COD$_{HPro}$) | – | HPro | |
| | | | | | 05:95 (%VS) | 5290 (COD$_{HPro}$) | – | HPro | |
| | | | 5 | 8 | 14:86 (%VS) | 2580 (COD$_{HPro}$) | – | HPro | |
| | | | 35 | | | 5450 (COD$_{HPro}$) | – | HPro | |
| | | | 50 | | | 6490 (COD$_{HPro}$) | – | HPro | |
| Oyster champost | Batch | | 30 | No | 75:25 (%TS) | – | 521 (COD) (VS) | HAc | (Fang et al., 2019) |
| | | | | | 50:50 (%TS) | – | 596 (COD) (VS) | HAc | |
| | | | | | 25:75 (%TS) | – | 505 (COD) (VS) | HAc | |
| FW | Batch | | 37 | 4.5[e] | 50:50 (%VS)[f] | 1350 (VFA) | 128 (VFA) (VS) | – | (Fernando-Foncillas and Varrone, 2021) |
| | | | | 6.8[e] | 50:50 (%VS)[g] | 1310 (VFA) | 222 (VFA) (VS) | – | |
| | | | | 9.0[e] | 50:50 (%VS)[f] | 1760 (VFA) | 218 (VFA) (VS) | – | |
| | CSTR | 4 | 37 | 9.0[e] | 20:80 (%VS) | ~11,000 (VFA) | ~200 (VFA) (VS) | HHex | |
| | | 2 | | | | ~8000 (VFA) | 130 (VFA) (VS) | HHex | |
| Paper waste | Batch | | 35 | No | 75:25 (%V) | – | 133 (COD) (TSS) | HAc | (Luo et al., 2021) |
| | | | | 5 | | – | 149 (COD) (TSS) | HAc | |
| | | | | 9 | | – | 196 (COD) (TSS) | HAc | |
| | | | | No | 50:50 (%V) | – | ~160 (COD) (TSS) | HAc | |
| | | | | 5 | | – | 208 (COD) (TSS) | HAc | |
| | | | | 9 | | – | 129 (COD) (TSS) | HAc | |
| | | | | No | 25:75 (%V) | – | ~175 (COD) (TSS) | HAc | |
| | | | | 5 | | – | 252 (COD) (TSS) | HAc & HBu | |
| | | | | 9 | | – | 98 (COD) (TSS) | HAc | |

[a] VFA$_{max}$ concentration numerator unit in brackets.
[b] Fermentation yield numerator unit in brackets.
[c] Fermentation yield denominator unit in brackets.
[d] Thermal pre-treatment at 50 °C during 8 weeks in the presence of air.
[e] The pH was only adjusted at the beginning of the experiment.
[f] Substrate to inoculum ratio (S/X$_o$) of 4.
[g] Substrate to inoculum ratio (S/X$_o$) of 8.

TS-basis (C/N ratio of 19.3), while the other two mixtures (25:75% and 75:25%) reached a similar VFA yield regardless of the C/N ratio (26.0 and 12.7, respectively). Fang et al. (2019) also reported that the COD$_{VFA}$/ sCOD ratio was constant for all mixtures and hypothesised that the non-VFA sCOD was either non-biodegradable or its fermentation was limited by the presence of humic-like compounds. The accumulation of non-VFA sCOD is a common feature in most publications although limited information has been provided about its composition. Further research is needed to characterise the non-VFA sCOD and to understand fermentation limiting factors to develop strategies that allow improving fermentation yields.

**5. Implementation of co-fermentation in WWTPs**

The integration of co-fermentation in a WWTP depends on the type of sewage sludge (PS, WAS, or combined) used as the main substrate and on the final use of the produced VFA. Fig. 2 illustrates the three main configurations considered in the literature to integrate co-fermentation in a WWTP: (i) co-fermentation to support biological nutrient removal (BNR), (ii) co-fermentation to produce polyhydroxyalkanoates (PHA), and (iii) co-fermentation for subsequent electricity production through microbial fuel cells (MFC). The configurations in Fig. 2 consider WAS as the main

substrate since WAS is the most studied main substrate in co-fermentation research. Alternatively, PS could also be considered as the main substrate for the co-fermentation process. All the configurations in Fig. 2 include an anaerobic digester to produce biogas from PS and the remaining solid fraction after co-fermentation.

*5.1. Biological nutrient removal*

Using the produced VFA to support mainstream BNR is the most straightforward configuration to implement co-fermentation in a WWTP (Fig. 2A). Indeed, some full-scale WWTPs have already implemented sludge mono-fermentation to support BNR (Jensen et al., 2017; Liu et al., 2018; Wang et al., 2019). In this configuration, the VFA produced are used as carbon source for denitrification and/or enhanced biological phosphorus removal, which reduces the use of external carbon sources such as methanol or ethanol (Gao et al., 2011; Long et al., 2014). Reducing the consumption of chemicals to support BNR is important to decrease the operating costs and environmental impacts of WWTPs.

Among the few studies related to the implementation of co-fermentation in WWTPs to support BNR, Long et al. (2014) reported that co-fermenting PS and grease trap led to a positive net present value as a
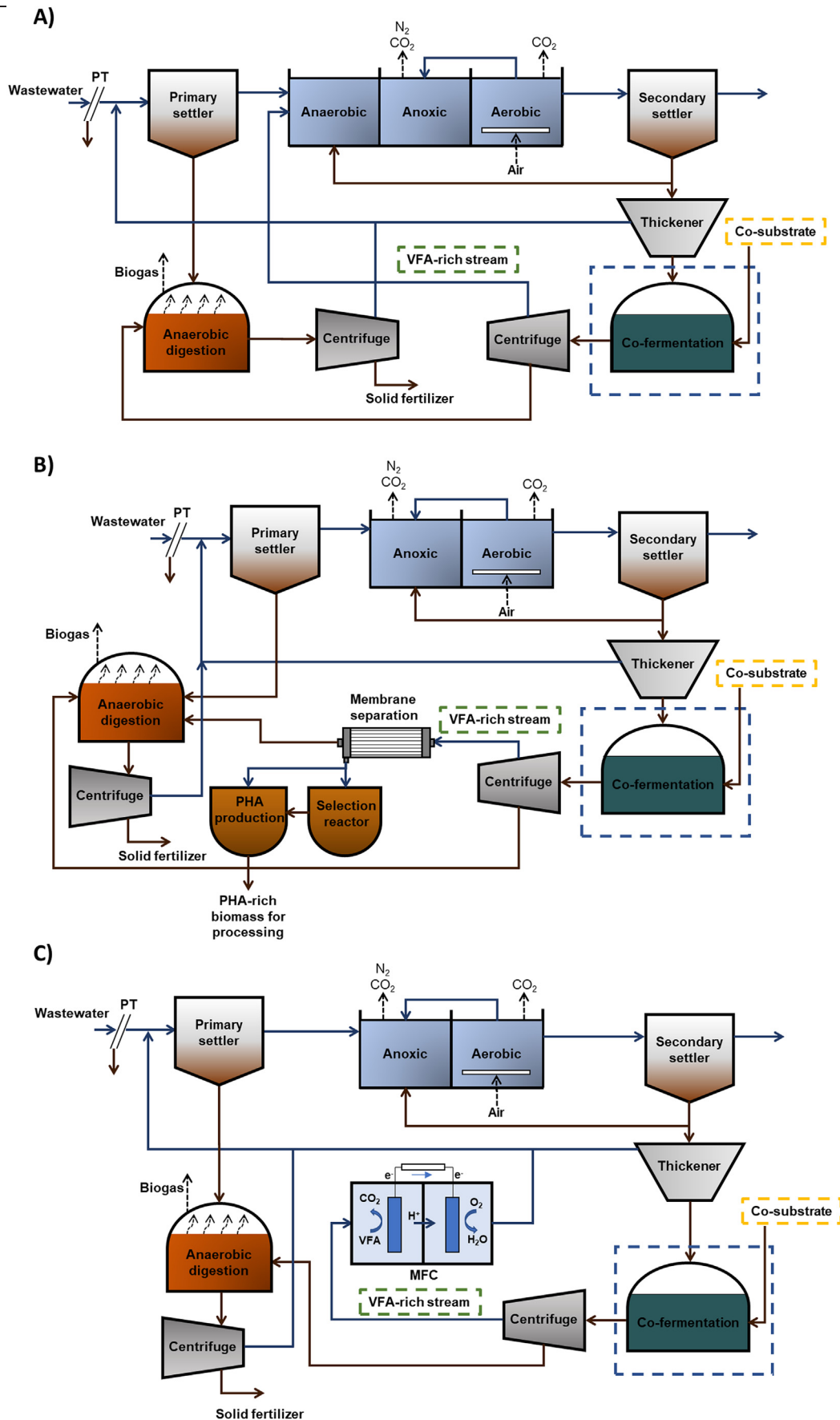
**Fig. 2.** Configurations to integrate co-fermentation in a WWTP: (A) co-fermentation to support BNR; (B) co-fermentation for PHA production and (C) co-fermentation for energy production through MFCs.

result of the reduction in methanol consumption. However, this study also observed that the high concentration of nutrients in the fermentation liquor could compromise the compliance of the phosphorus discharge limit. Grease trap is expected to have a low nutrient content, however, the solubilisation of nitrogen and phosphorus from the sludge increases the amount of these compounds to be treated in the BNR (Feng et al., 2011; Gao et al., 2011). This is particularly important for phosphorus considering the stringent discharge limits for this compound. However, the impact of nitrogen and phosphorus solubilisation during sludge fermentation on the overall WWTP nutrient balance is expected to be neutral since, without co-fermentation, these nutrients would have been solubilised in the anaerobic digester and recycled back to the mainstream of the WWTP. However, it could be argued that the amount of phosphorous removed through uncontrolled precipitation (e.g., struvite, calcium phosphate) is lower in sludge fermentation than in sludge anaerobic digestion due to the lower pH in fermenters (Feng et al., 2011; Latif et al., 2017). Further studies are needed to evaluate the impact of co-fermentation on the WWTP nutrient balance and effluent quality.

The use of co-fermentation to support BNR should consider the need for external biodegradable organic carbon. Co-fermentation could be particularly suited for those WWTPs with high consumption of chemicals for BNR since the high VFA yield reached in the co-fermenter may offset a large fraction of the WWTP operational costs. However, the implementation of mono-fermentation may be sufficient in WWTPs with low requirements of external organic carbon. Accordingly, techno-economic studies are necessary to evaluate under which conditions the implementation of co-fermentation to support BNR is economically attractive for WWTP operators. Importantly, these studies should consider not only the potential of the co-substrate to improve VFA yield but also the nutrients backload in the VFA-rich stream and the biogas production in the anaerobic digester, among others.

### 5.2. PHA production

The production of PHA is another alternative to implement co-fermentation in a WWTP (Fig. 2B). In this configuration, the VFA-rich stream from the co-fermenter is used as a feedstock to produce PHA. The production of PHA consists of two stages: (i) a first stage, where the PHA-accumulating organisms are selected (selection reactor) and (ii) a second stage, where the intracellular accumulation of PHA is maximised (accumulation reactor) (Mannina et al., 2020). After the accumulation stage, the PHA-rich biomass is processed for PHA extraction and purification. A preconditioning of the VFA-rich stream (e.g., centrifugation and membrane filtration) is needed to remove suspended solids that could reduce the purity of the recovered PHA (Moretto et al., 2020).

Moretto et al. (2020) co-fermented WAS and FW in a pilot-scale plant to produce VFA for subsequent PHA production. Their results showed that the implementation of co-fermentation allowed increasing the carbon load to the PHA system, which led to higher PHA yields in the selection and accumulation reactors when compared to studies using lower carbon loads. These results indicate that co-fermentation could be a strategy to maximise the yield and profit from PHA production in a future WWTP. Moretto et al. (2020) also reported that the revenues obtained from WAS-FW co-fermentation followed by PHA production were higher than those obtained from WAS-FW anaerobic co-digestion. However, the economic analysis did not consider the capital and operating costs associated with the implementation of co-fermentation and PHA production (e.g., co-fermentation reactor, pre-conditioning of the VFA-rich stream, PHA selection and accumulation reactors, and downstream processing for PHA extraction and purification), which could have a large impact on the economic feasibility of PHA production.

### 5.3. MFC energy production

The use of MFC to recover energy from VFA is another reported alternative to implement co-fermentation in a WWTP (Fig. 2C). In MFC, the VFA

are bio-oxidised in the anode while producing electricity (Chen et al., 2013a; Teng et al., 2010).

Chen et al. (2013a) evaluated the efficiency to recover electricity through MFCs from the VFA-rich stream produced from WAS-FW co-fermentation. Their results showed that the production of electricity was significantly enhanced when using the co-fermentation liquor compared to the WAS mono-fermentation liquor. The authors concluded that the co-fermentation liquor was suitable to produce electricity by means of MFCs. However, MFC technology is not yet economically feasible since the energy produced hardly offsets the capital costs of MFC implementation (Trapero et al., 2017). Conversely, other well-established alternatives such as anaerobic co-digestion are nowadays more attractive for WWTP operators to increase electricity production with relatively low capital and operating costs (Macintosh et al., 2019; Vinardell et al., 2021).

### 6. Knowledge gaps and future research

There is a limited number of publications in the peer-reviewed literature studying waste co-fermentation to produce VFA, lactic acid and alcohols (albeit lactic acid and alcohols are not always reported). Accordingly, there are several topics and knowledge gaps that need to be further researched.

The highest WAS-FW co-fermentation yields have been achieved at circumneutral pH. However, due to the acidic nature of fermentation, operating a fermenter at circumneutral pH would require equipping the fermenters with pH control systems. Garcia-Aguirre et al. (2019) stated that these control systems are complex and can lead to operational problems. Operating the fermenter without pH control relying on the WAS buffer capacity (self-regulated pH) or adjusting the pH of the influent to alkaline values (pH 9–10) have been used in several co-fermentation studies. However, the pros and cons of the different pH control strategies on fermentation yield, product profile, process controllability/stability and economic feasibility remain unknown.

The impact of temperature on co-fermentation performance has received limited attention. Most experiments have been carried out at mesophilic conditions (35–37 °C), although optimum performance has also been reported at psychrophilic (20–25 °C) and thermophilic (50–60 °C) conditions. The disparity between these publications could be related to the fact that most experiments have been carried out in batch, with the methodology and set-up of these batch assays varying widely among publications. Batch assays are an excellent screening tool but have limitations. Batch results are driven by the capabilities of the starting microbial community and do not allow evaluating the microbial acclimation to different operational conditions and substrate composition that occur under continuous operation. Furthermore, a standardised protocol for fermentation batch assays should be developed, tested and validated to facilitate experiments' comparability and reproducibility. This protocol should emphasise the importance of replicates as well as blanks and controls test.

Fermenters' operational conditions need to promote hydrolytic and fermentation activity while limiting the proliferation and activity of methanogens. However, neutral pH and mesophilic conditions are ideal conditions for methanogenic archaea. The proliferation of methanogens in CSTR could be limited by operating at low HRTs; however, the HRT should also be large enough to allow sufficient waste fermentation. Methanogenic activity has been recorded at HRT as low as 2 days (Fernando-Foncillas and Varrone, 2021; Ho et al., 2014; Long et al., 2014; Peces et al., 2021), indicating that the HRT itself may not be enough to prevent the proliferation of methanogens in fermenters. Temperature, pH and/or OLR are variables that can be easily manipulated to limit the presence of methanogens in the fermenter. The influence of these factors can only be studied under continuous conditions. In continuous experiments, the microbial community will evolve and adapt over time to the operational conditions, therefore, the fermenters should be operated long enough to reach steady-state conditions (minimum of 3 HRT-equivalent). Similar to any other monitoring parameter, microbial communities may show some

variability over time and several independent samples are required to characterise the steady-state conditions.

Analysing the microbial communities at the end of batch assays has been done in some publications. However, generalised conclusions from microbial community analyses in batch assays are strongly discouraged since results are largely influenced by the starting microbial community. It should be considered that batch assays are non-stationary experiments, therefore, the microbial community will change over time as the experiment evolves. A clear example is the recurrent consumption of acetic acid after ~7 days of batch, which can be attributed to the growth of methanogenic archaea. Accordingly, the microbial community at the end of the test may not reflect the community during the most active period of the batch (generally the first 3 days). It is recommended that conclusions from microbial community analyses are obtained from well-characterised continuous experiments under steady-state conditions.

There are other factors such as the proportion between main substrate and co-substrate, the impact of the co-substrate composition and pretreatment, or using PS as main substrate (instead of WAS) that have received limited attention. This information is important to help WWTP operators choosing the main substrate (PS and/or WAS) as well as selecting the origin and the amount of the co-substrate. The consumption of acetic acid during fermentation is recurrent in many studies (both batch and continuous) when using WAS as the main substrate, which has been primarily related to the presence of methanogens in the fermentation liquor. Additionally, WAS also introduces denitrifying bacteria, sulphate-reducing bacteria (SRB) and phosphorus accumulating organisms (PAO), which are all VFA consumers (Nierychlo et al., 2020). The relative abundance of these microorganisms in PS is smaller, which may facilitate the accumulation of acetic acid in the fermentation liquor.

Finally, based on knowledge gained through this literature review and the authors' expertise in the field, a series of general recommendations are given to provide a more uniform framework in future publications as well as to facilitate studies cross-comparison. Multiple units have been used to establish mixing ratios between substrates (e.g., wet, volume, TS, VS, VSS, COD). Considering that fermentation performance is linked to the amount of organic matter fed to the system, it is encouraged that authors report mixtures on a VS or COD basis. This does not imply that authors cannot report additional units based on their needs and research goals. The fermentation products concentration ($mgCOD \cdot L^{-1}$) and yield ($mgCOD \cdot gVS^{-1}$) should be reported in COD to account for the different energy densities of the different molecules. VFA, lactic acid and ethanol concentrations can be converted from mass to COD-equivalents using the theoretical value based on their elemental composition ($mgCOD \cdot mg^{-1}$): 1.07 for acetic acid, 1.51 for propionic acid, 1.82 for butyric acid, 2.04 for valeric acid, 2.21 for caproic acid, 2.34 for heptanoic acid, 1.07 for lactic acid and 2.09 for ethanol. Finally, not all publications provided enough information to understand how the experiments were set-up and/or monitored, including important parameters such as (i) pH values, despite its importance in fermentation, (ii) set-up of mixtures and blanks, (iii) oxygen exposure and $N_2$ flushing, (iv) mixing conditions and feeding regime, and (v) procedure and methodology used to monitor fermentation products.

## 7. Conclusions

Co-fermentation is an opportunity for WWTPs to increase the sludge mono-fermentation yields and, to a certain extent, drive the product profile. Primary sludge and waste activated sludge have been successfully co-fermented with a wide variety of co-substrates such as food waste, paper waste, crop residues, and fat, oil and grease. Research has been primarily carried out using batch assays, whereas a low number of publications have evaluated co-fermentation under continuous conditions. The highest fermentation yields have been generally achieved when the mixture was dominated by the most biodegradable substrate at circumneutral pH and mesophilic conditions. However, the limited number of publications and the disparity between experimental methodologies do not allow providing guidelines for optimum mixing ratio and operating conditions. Finally, the

technical and economic implications of co-fermentation in a WWTP should be holistically evaluated considering VFA production, the supported biotechnologies (e.g., biological nutrient removal, PHA production), and the carbon and nutrient (N and P) balance of the WWTP.

## CRediT authorship contribution statement

**Noemí Pérez-Esteban:** Conceptualization, Formal analysis, Investigation, Data curation, Writing - original draft, Visualisation. **Sergi Vinardell:** Investigation, Writing - original draft, Visualisation. **Carme Vidal-Antich:** Investigation, Data curation, Writing - review & editing, Visualisation. **Miriam Peces:** Conceptualization, Data curation, Writing - review & editing, Visualisation. **Sergi Peña-Picola:** Investigation, Writing - review & editing. **Josep Maria Chimenos:** Writing—review & editing, Funding acquisition. **Joan Dosta:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition. **Sergi Astals:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.152498.

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