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Potential and limitations of co-fermentation: A review

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Every day we know more and understand less.

Albert Einstein

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ABSTRACT

Fermentation is a biotechnological process to generate value from organic waste. During this process, volatile fatty acids (VFA) are obtained as a product, which can be directly used to support other biotechnologies and contribute to the circular economy. However, some substrates present a series of characteristics that are not totally optimal. Co-fermentation is a way to alleviate these drawbacks, which consists on fermenting two or more substrates simultaneously. Co-fermentation is a relatively new approach to the fermentation process as the articles ranging from 2013-2020 account for 77%. A wide range of substrates and combinations have been studied. The more utilised main substrate is waste activated sludge (WAS) followed by primary sludge (PS). Most publications have focused on studying the combination of WAS and food waste (FW) and WAS and agro-industrial (e.g. corn stalk and mushrooms). Most researchers emphasize pH control using chemicals and balancing C/N ratio. Besides, the substrate has been shown to influence the VFA profile as well as the pH. The addition of agro-industrial residue can delay the co-fermentation process due to its high content in lignocellulosic compounds. Overall, other parameters and mixtures should be studied, and more continuous experiments are needed to finish studying co-fermentation.

Keywords: circular economy; biorefinery; fermentation; co-fermentation; lactic acid; volatile fatty acids (VFA)

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NOMENCLATURE

AD	Anaerobic Digestion
AI	Agro-industrial
BNR	Biological Nutrients Removal
COD	Chemical Oxygen Demand
EPS	Extracellular Polymeric Substances
FW	Food Waste
FOG	Fat Oil and Greases
HRT	Hydraulic Retention Time
IWW	Industry Wastewater
OFMSW	Organic Fraction of Municipal Solid Waste
MEF	Managed Ecosystem Fermentation
MS	Mix Sludge
PAO	Polyphosphate Accumulating Organisms
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PHBV	Polyhydroxybutyrate-valerate
PHV	Polyhydroxyvalerate
PM	Pig Manure
PS	Primary Sludge
RRF	Resource Recovery Facilities
SCFA	Short-Chain Fatty Acids
SH	Slaughterhouse
SRT	Sludge Retention Time
SS	Sewage Sludge
TAN	Total Ammoniacal Nitrogen
TS	Total Solid
VFA	Volatile Fatty Acids
VS	Volatile Solid
WAS	Waste Activated Sludge
WWTP	Wastewater Treatment Plant

1. INTRODUCTION

The current economic dynamics requires more sustainable and renewable technologies to maximize resource recovery from waste streams. For this reason, waste treatments plants are being transformed into resource recovery facilities (RRF). Anaerobic biotechnologies are crucial to accomplish this goal since they comprise a wide range of microbially-driven technologies able to transform the organic-rich solid waste and wastewaters into high-value products such as methane, hydrogen and volatile fatty acids [1]. RRF are also known as biorefineries, that integrate a network processes from which transform biomass and residues into high-value products [2]. Mixed culture fermentation is a key process in most biorefineries, because it can transform waste into volatile fatty acids (VFA), lactic acid and alcohols. Besides their commodity value, VFA can be directly used to support other biotechnologies such as biological nutrient recovery, bioplastics production and chain elongation.

Fermentation is an anaerobic biological process that breaks down organic matter into easily assimilable compounds, e.g. VFA for acidogenic fermentation, lactic acid as a product of lactic fermentation and alcohols for alcoholic fermentation [3]. During waste fermentation process three stages can be differentiated: (i) hydrolysis, (ii) acidogenesis (primary fermentation) and (iii) acetogenesis (secondary fermentation). To promote the accumulation of volatile fatty acids, fermentation technology requires the suppression of the methanogenesis stage of the anaerobic degradation process.

During the hydrolysis, the hydrolytic-fermentative bacteria breaks down the organic matter particles (i.e. proteins, carbohydrates and lipids) into soluble monomers (amino acids, poli- and mono-saccharides, glycerol and long chain fatty acids (C_{12}-C_{22})). Subsequently, acidogenic bacteria (acidogenesis) transform the hydrolysis products into VFA including valeric acid (C_5), butyric acid (C_4), propionic acid (C_3), and acetic acid (C_2). Lactic acid (C_3) can also be produced from waste fermentation. In this work, the sum of VFA and lactate is referred as short-chain fatty acids (SCFA). Acidogenesis also produces hydrogen gas, carbon dioxide and pyruvate among others. Finally, acetogenesis convert valerate, butyrate and propionate into acetate (Figure 1). Hydrogen is produced in all fermentation reaction since it acts as electron sink.

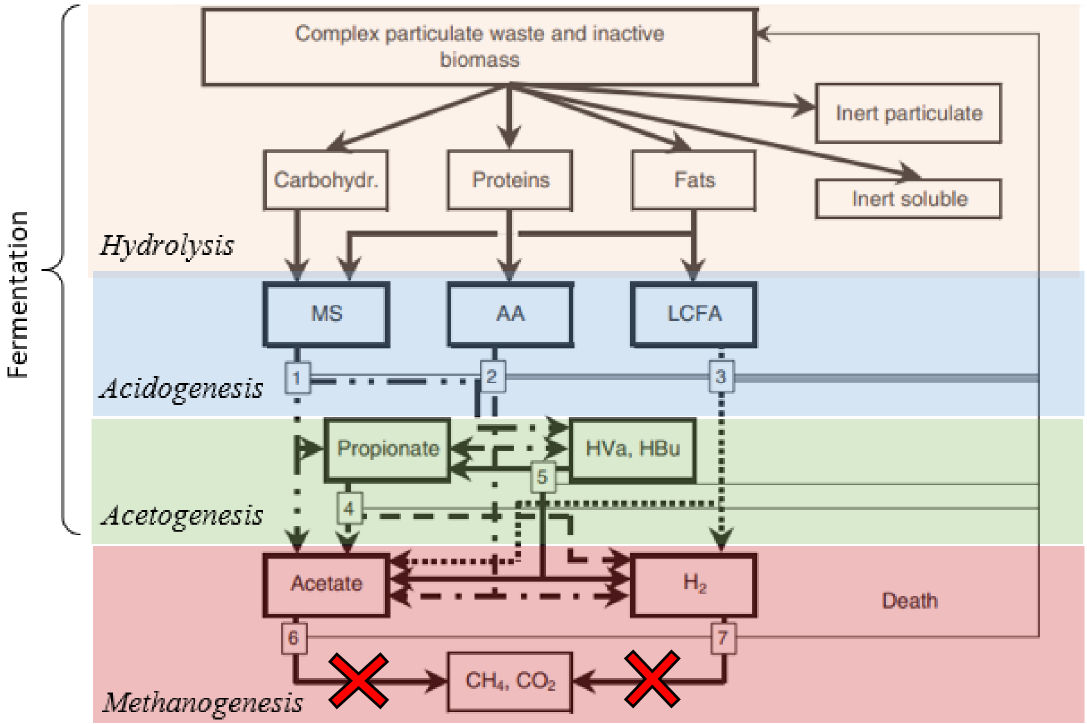

Figure 1. Anaerobic model as implemented including biochemical processes. (Adapted from [4])

1.1.Application of VFA

Volatile fatty acids (VFAs), the main product of fermentation, are short carbon-chain (six carbon or less) organic acids like formic, acetic, propionic, butyric, valerate and caproic [5]. VFA are classified as water-soluble fatty acids that can be distilled at atmospheric pressure [5]. In a biorefinery scenario, VFAs are used to provide the easily available carbon source needed for other microbially-mediated biotechnologies, such as polyhydroxyalkanoates (also known as bioplastic) and biological nutrient removal (BNR) and nutrient recovery biotechnologies [1]. However, each application requires a the specific VFA or VFA pool. Finally, it is worth highlighting that VFA extraction and purification from the fermentation liquor is not addressed in this literature review since there are literature reviews devoted to this topic in the literature [6–9]. Purified acids have a marked value: acetic acid 600 $\$\cdot t^{-1}$, propionic acid 2,000 $\$\cdot t^{-1}$ and from butyric acid 2,163 $\$\cdot t^{-1}$ in 2015 [10].

1.1.1. Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHA) is a biodegradable family of polymer made by microorganism using VFA as a carbon source [11]. Using feast/famine strategy, carbon source is stored inside as a biopolymer. The difference between the petrol plastics and PHA is that the latter is biodegradable, bio-compostable and comes from a renewable source [12]. PHA can be classified in two co-polymers: polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV). Importantly, the bioplastic obtained from the process is highly dependent on the VFA used to feed the PHA-accumulating organisms. Acetate and butyrate are precursors of PHB, whereas propionate and valerate are precursor of PHV [13,14].

1.1.2. Biological nutrient removal

VFAs are employed as carbon source for nitrogen and phosphorus biological removal/recovery from wastewater treatment plants (WWTP). In addition, this VFA can be obtained from the same plant by fermenting primary sludge (PS), waste activate sludge (WAS) and/or their combination (known as mixed sludge or sewage sludge) [15,16].

VFA from waste fermentation is an economic solution for WWTP denitrification [17]. However, there is differential performance of BNR depending on the type of the VFA. For the nitrogen removal process, denitrifying bacteria have preference for lower molecular weight VFA. Acetate is normally the first VFA to be consumed, followed by propionate and butyrate, and lastly valerate [16]. The average specific denitrification rate for acetate is two times higher than that of propionate [18].

VFA also needed by polyphosphate accumulating organisms (PAO) which store VFA as PHA under anaerobic condition, which later, under aerobic conditions, is used to biomass growth and phosphorous uptake (feast/famine strategy). PAO have the capacity to accumulate larger amount of phosphorus than other microorganisms. VFA profile is important since PAO prefer propionate and lactate instead of acetate [19].

1.1.3. Other products

There is a wide range of uses for volatile fatty acids, but they are not addressed in this literature review as there are already literature reviews dedicated to these topics: biohydrogen [20,21], biomethane [22], biological sulphate reduction [23], microbial fuel cell [24,25] and biodiesel [26,27].

1.2.Co-fermentation

1.2.1. Limitations of fermentation

An organic biodegradable substrate is needed to carry out fermentation. To-date, a wide range of organic-rich waste streams have been used to produce VFA through fermentation, including primary sludge (PS), waste activated sludge (WAS), animal manure (mostly pig manure PM), micro- and macro-algae, food waste (FW), fat oil and greases (FOG), slaughterhouse waste (SH) and agro-industrial residues (AI). Among them, PS, WAS and FW are the most utilised waste.

Primary sludge and WAS are produced in large amounts in wastewater treatment plants (WWTP). Both sludges are relatively rich in total carbon oxygen demand (COD) with COD concentration ranging 58-81 and 11-58 $gCOD\cdot L^{-1}$, respectively [14,28–30]. Nevertheless, the soluble COD concentration is ten to hundred times lower than its total COD [31]. The high concentration of particulate COD (total COD minus soluble COD) makes hydrolysis the limiting step [31]. Depending on the fermentation conditions, the VFA yield for primary sludge can vary between 19-600 $mgCOD\cdot gVS^{-1}$ [29,32–34], while the VFA yield for WAS ranges between 10-24 $mgCOD\cdot gVS^{-1}$ [35–39].

The VFA yields reported for FW are higher than for sludge, with value ranging 28-971 $mgCOD\cdot gVS^{-1}$ [38,40–43]. It is well known that FW have low nutrients and low buffer capacity [44], which may limit fermentation performance. Moreover, FW may contain a high content of lignocelluloses that may be difficult to degraded [25]. PM is also produced in large quantities, but its production is more localised than sewage sludge and food waste. PM is characterized by a high ammoniacal nitrogen concentration (2.0-5.5 $gN\cdot L^{-1}$ [45,46]). As a result, a low C/N ratio is obtained, which is not optimal for fermentation [47,48]. Microalgae (microscopic unicellular algae) and macroalgae (multicellular macroscopic algae) are an emerging substrate for anaerobic digestion and fermentation. First publications regarding algal fermentation have been recently published [26,49]. Macroalgae's are usually contain abundant lignocellulosic carbohydrates [50], while microalgae are rich in protein [50]. FOG corresponds to the 25–40% of the municipal sewage total COD and it is usually removed in the aerated grit chamber of the WWTP (50–90%) [51]. Also, FOG is collected in grease traps from restaurants and food processing industry. FOGs consist mostly of lipids which not have enough moisture, and essential nutrients to ferment by themselves [28].

SH fermentation has not received much attention over the years in terms of fermentation. There are a wide range of waste of this industry, blood, meat and bone meal, wastewater and rumen. In case of blood, it has a low total solid content with a high content of protein [49]. As far the meat and bone meal and the wastewater, the protein content is high [52]. It has positive characteristics like nutrients content or buffer capacity [53]. Rumen have a huge microbiota content but the optimal pH for them is between 5 and 7 [54]. Finally, it is important to keep in mind that crops and agro-industrial wastes are seasonal substrates, which might deficiency N and low alkalinity [55].

1.2.2. Opportunities of co-fermentation

Co-fermentation is based on fermenting two or more substrates to improve VFA yield and drive the fermentation profile. Co-fermentation aims to improve the limitations that substrates of mono-fermentation by: (i) increasing in organic content; (ii) diluting of inhibitory and/or toxic compounds; (iii) balancing the C/N ratio; (iv) reducing of reactor volume; (v) improving the of buffer capacity (procure a constant pH); (vi) providing an active fermentative microbial community and (vii) promoting synergistic effects in VFA production [28,51,56–58].

The waste organic concentration is crucial to accomplish the fermentation process. The more content in organic matter, more matter would be likely degraded by fermentative bacteria. For instance, PS, WAS and PM are quite diluted and their total solid (TS) concentration around 3-5% [28,59]. However, organic matter is not the only factor to take in consideration, some substrates may contain microbial inhibitors or toxic compounds, however, co-fermentation dilution could decrease inhibitors concentration making less harmful to the fermentation process. An optimum carbon-nitrogen ratio is essential for the microorganism anaerobic fermentation growth [57]. Besides, variation of C/N ratio may result in different profiles of VFA [55]. Therefore, substrates with a nitrogen deficiency can be fermented together with those that have nitrogen abundance, for example, WAS-FW [52,59,60], PM-FW and WAS-FOG [28]. The combination with substrates with high alkalinity prevents pH changes during the process. Indeed, the fermentation media buffer capacity is one of the key factors of fermentation since fermentative bacteria cannot survive below pH 3 [37,61]. Additionally, pH of the fermentation process controls the VFA profile [62,63]. The best-known co-fermentation example is the combination between WAS and FW, where the WAS provides buffer capacity to the FW, avoiding strongly acidic pH.

As it is mentioned before, some potential substrates like FOGs do not have the capacity to self-sustain the microbial community required to carry out fermentation. For this reason, providing active fermentative microbial community is crucial to ferment waste than present low fermentative activity, e.g. FOG and agricultural waste. Finally, there is an economic benefit on centralizing waste treatment due to the better utilization of the future fermentation infrastructures.

Overall, co-fermentation stands as an opportunity since it allows us to overcome the fermentation deficiencies of different substrates and limit operational costs associated to the purchase or chemical reagents, while boosting fermentation VFA yields.

2. OBJECTIVE

The goal of this master project is to conduct a literature review on co-fermentation experiences of a wide range of substrates. This work aims to identify potential scenarios and as well as knowledge gaps from which develop future lab-based research and applications.

3. LITERATURE REVIEW

3.1. Data collection

A literature search was made on co-fermentation based on their publication date, using Scopus, Web of Science and Google Scholar. Following this technique at the time of reading, changes were appreciated in the paper's introduction. During these weeks, a summary of the co-fermentation literature has been carried out. Through this process, a summary table has been filled, including the set-up, VFA yields, VFA profile as well as the main and remarkable findings. These features are found in Table 1. As Table 1 shows, the features were divided into 4 groups. First one is the identification of the paper, the authors, the year of publication, the title and the URL. Operation conditions were later recorded. Publications were grouped based on substrate type, temperature conditions, fermenter type among others. Experimental results were collected from both mono- and co-fermentation conditions. Finally, a summary table (Table 1) was filled up with the most interesting findings of the publications.

Table 1. Important characteristics to consider of the literature

Identification	Operation Condition		Mono- and co-fermentation	
Author	Type of assay	Batch/Continue	pH	-
Title	Temperature	ºC	[VFA]	mg[a]·L^{-1}
Year	HRT and SRT	day	Yield $_{VFA}$	mg[a]·g[b]$^{-1}$
URL	Buffer	Type	Proportion VFA	%[a]
	Main and Co-Substrate	-	TAN	mg $N\text{-}NH_4^+ \cdot L^{-1}$
	Mixture	%VS, volume…		
	Pre-treatment	-		

[a] VFA or COD

[b] COD or TS or VS or VSS

3.2.Review outline

As shown Fig. 2, co-fermentation is an emerging topic of research. It can be seen that the publication of papers started to be significant relatively recently (2013). In 2016, 2019 and 2020, were the years with more articles, which represents 13% each other.

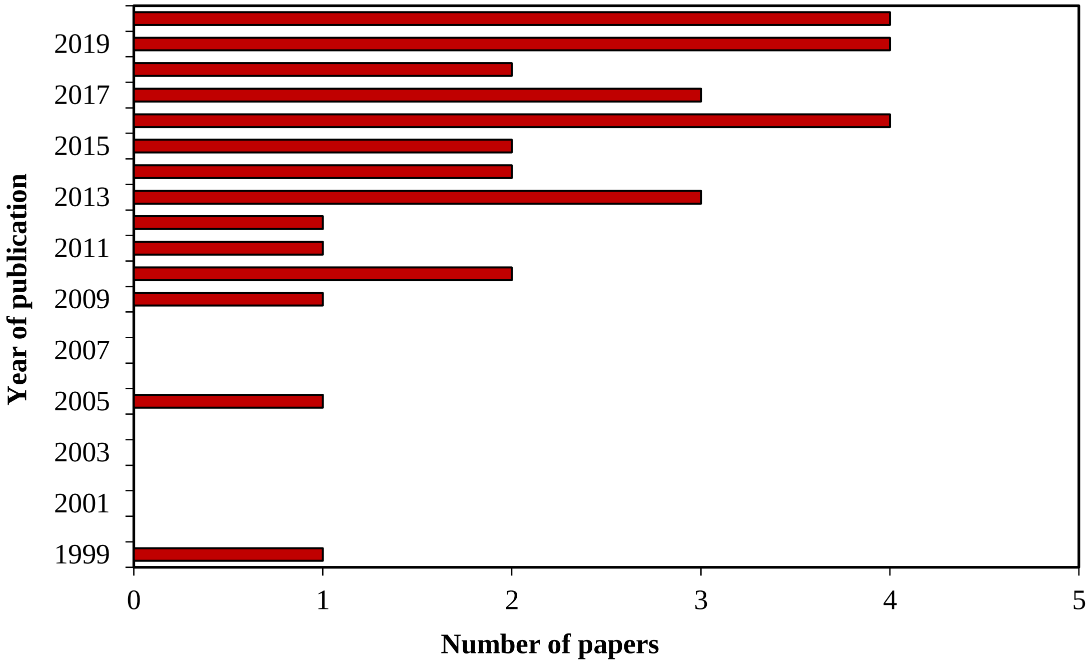

Figure 2. Evolution of published co-fermentation articles found

All the articles dealing with co-fermentation have been read and studied. Figure 3 shows that the dominant main substrate was WAS and FW as co-substrate. This mixture corresponds approximately to one third of the papers found (12 out of 31 papers). Next, is the combination of WAS with agro-industrial (AI) with 7 papers. PS is the second most used substrate in co-fermentation without a clear dominant clear co-substrate.

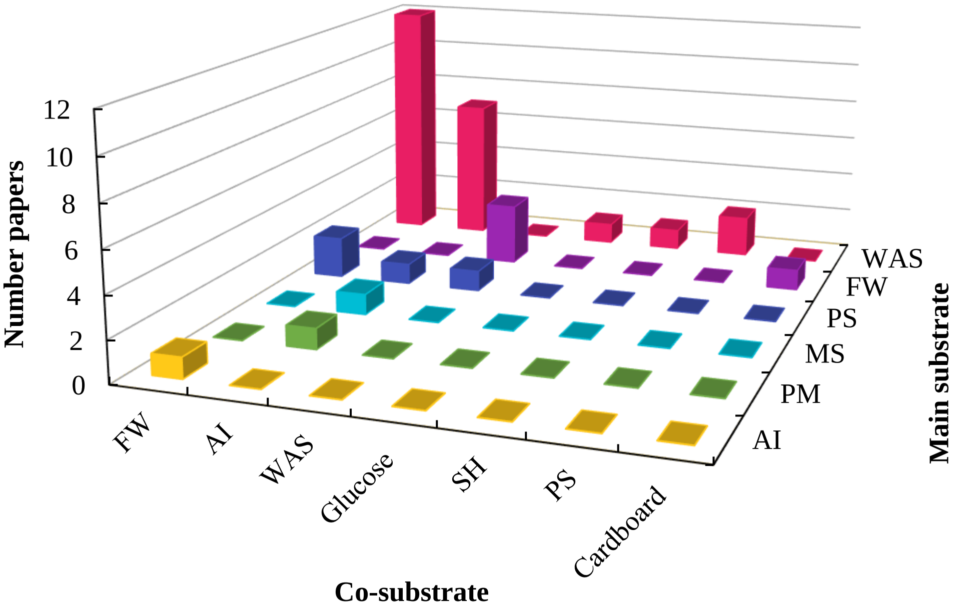

Figure 3. Main substrates and co-substrates in co-fermentation papers found.
(AI: Agro-industrial, FW: food waste, IWW: industry wastewater, MS: mix sludge, PM: Pig manure, PS: Primary sludge, SH: Slaughterhouse, WAS: waste activated sludge)

The papers could be divided in two groups depending on the focus of the introduction. One the one hand, there were the articles of 1999 to 2004, which were focus on the production of hydrogen or biogas. On the other hand, the articles from 2005 onwards are devoted to VFA production and biological nutrient removal. The change of tendency is caused by the yield obtained in these two products. Hydrogen yield (4-290 $molH_2 \cdot gVS^{-1}$) [64–69] is less than VFA yield of the sludges (mentioned in *section 2.1*). Also, it is important to highlight the techno-economic analysed. The price of H_2 2.29 \$ per m^3 [70] is lower than VFA (mentioned in *section 1.2)*. In fact, recovery of VFA is focus on butyric because is the VFA with the higher price in the marked, obtained a profit of 296 $\$ \cdot t^{-1}$ [71]. Calt et al. [10], estimated that for 1 tone of organic waste using Managed Ecosystem Fermentation (MEF) with a 100% of separation of VFA the 35% in weigh were VFA with 2,775 $\$ \cdot t^{-1}$.

It also seen that the composition of FW is very variable depending on the origin and the country where the research is performed. Finally, it is worth mentioning that the total ammoniacal nitrogen (TAN) is not considered in many papers. It is important because it gives a knowledge of the hydrolysis (first step of fermentation). In other words, indicated the level of protein degradation. Theoretically, the more ammonia in the fermentation liquid, the higher VFA production. [72,73]. In addition, the presence of the nitrogen can affect the metabolic pathway of bacteria and therefore the profile of VFA [55].

3.3.Waste activated sludge as a main substrate

Waste activated sludge (WAS) stands as the most used main substrate for co-fermentation. The most used combination in this process is mixing with FW. Although, WAS has been co-fermented with other substrates like agro-industrial (e.g. mushrooms and corn straw), primary sludge, glucose and slaughterhouse waste.

Using WAS as a substrate provides positive aspects to co-fermentation. WAS has a buffering capacity that allows microorganism not to inhibited by a low pH [74]. It can provide enough moisture to those substrates that have a high solid concentration [58]. Besides, WAS can provide an active microorganisms in co-fermentation to produce VFA [30]. Apart from that, WAS has a high organic compounds which is partly biodegradable (60-80% in dry weight) [41,75].

3.3.1. WAS co-fermentation with food waste

The term of FW in this review englobes different type of food residues such as rice, organic fraction of municipal solid waste (OFMSW), canteen waste, as well as synthetic food waste. The literature is focus on different mixture, pH and how specific type of food waste influence in the co-fermentation (Table 2).

As it is mentioned before, FW has a low buffer capacity which by combination with WAS can be solved. It has been seen that the purpose of the research was not this as most of them controlled the pH, but to maintain an optimal nutrient balance (C/N ratio).

WAS and FW co-fermentation have been successfully carried out in several studies. Feng et al. [40], co-fermented WAS and FW (2:1 in weight) at different pH. Their experiments showed that the co-fermentation produce more VFA than mono-fermentation (WAS only and FW only). The optimum pH range was between 6-9. Ma et al. [39], studied different mixtures WAS with synthetic FW and with potato peel waste as starch, at pH 10 and temperature of 35 ºC focusing on the influence of macromolecules. Also, Ma et al. [39] carried out thermal and alkali pre-treatment of WAS is done to release liberate extracellular polymeric substances (EPS) trapped in the WAS matrix. The experiment showed that, in case of the starch (potato peel waste) the concentration of butyric increase, whereas, lipids and protein provide significant concentration of propionate and valerate. It should be noted that the predominant acid in both mono- and co-fermentation was acetic acid. Li and Li [76] also evaluated the influence of different macromolecules from different type of food waste, for example: wheat, corn and rice for carbohydrates, chicken for proteins, and bean as carbohydrate with a high content of cellulose, under temperature of 35 ºC with no pH control. In terms of VFA concentration, wheat, corn and rice, are the most productive co-substrate. It has been seen that the chicken (proteins) and beans present more buffer capacity than the other co-substrates but obtained the low VFA concentration. In addition, these two substrates have a higher acetate concentration, while the rest co-substrates the dominant VFA was the propionate followed by acetic acid which was preferable for the authors as it is wanted to recover the phosphorus. Other authors such as Moretto et al. [77] studied different temperatures to carry out co-fermentation of WAS and FW. Thermophilic temperatures (55 ºC) contributes the solubilization of COD but a lower efficiency to transform it into VFA. Also, the experiment was done under mesophilic condition (37 ºC). For each temperature, different pH was studied (without pH control, pH 7, and pH 9). Additionally, for mesophilic conditions, thermal pre-treatment was carried out to assess the possibility to improve fermentation performance. The pre-treatment reduced the lag of 5 days to generate the VFA. Acetic acid was the main VFA, but in case of alkaline condition (9) predominated the propionic acid whereas in the acidic condition butyrate and caproic acid predominated. Some authors focused only on one type of food waste. In this case rice as it is the predominant residue in south-east Asia. Wu et al. [78] is one of the authors that co-fermented rice with WAS. This type of food waste is well-known by the high content of carbohydrates (80.5% dry weight). This author was carried out different mixtures, 10%, 20% and 30% of rice in dry weight under different acids pH, (without pH control, pH 3, pH 4 and pH 5) at a 35 ºC because they focused on the BNR. They were able to see that the proportion of 30% of rice at both pH 5 and uncontrolled (approximately 4.5) have a highly VFA concentration. For this reason, the best option was the uncontrolled pH since it did not suppose an economic expense. Rice was also used by Feng et al. [79] as a representation of FW. The mixture between WAS and rice was in C/N ratio basis, specifically 20. The maximum VFA yield was between a pH of 7-9. Moreover, the high presence of propionic was related to WAS due to the fact that carbohydrates promote the consumption of WAS protein.

Obtaining high lactic acid production and then obtaining propionic acid for BNR has been the goal for Li et al. [44] and Li et al. [59]. In a subsequent study, Li et al. [44] employed a two stages process. A first stage for maximizing lactic production and then, the second stage, to transform it into propionic acid using a pure culture. Through the study of pH, mixed ratio and temperature, optimal conditions were reached as pH 8,

VS_{WAS}-VS_{FW} ratio 1:6, a fermentation period for 2.5 days and at 20 ºC. Li et al. [59] was focus on the production of lactic to improve the propionic yield. For this reason, the study had to stages like previous one. The batch assay was done with 0.24:1 in grams (WAS:rice) at pH 8 under different temperatures. The maxim production of lactic was obtained at high temperatures 55 ºC and 65 ºC. In case of 35 ºC, the lactic produced was consumed quickly to obtain VFA, in other words, it is a precursor in the metabolic pathway.

FOGs are another type of organic waste that can be found in food waste in both household and restaurants waste. However, it can also be found in WWTP, industries and slaughterhouses. Peces, et al. [28], used oleic acid as a model of FOG. The author did different mixtures between WAS and oleic acid under 20 ºC with no pH control in semi-aerobic condition. It could be seen that co-fermentation helps the degradation of oleic acid by just 4.7%. The VFA concentration was higher in co-fermentation than in mono-fermentation of WAS. Moreover, the VFA profile changed thus increasing the butyric acid concentration and decreasing the caproic in co-fermentation. Zhao, et al. [80], studied the influence of salinity (NaCl) in FW in co-fermentation with WAS. The presence of salt accelerated the solubilization of FW and promotes the transformation of the protein into VFA. Besides, a low concentration of NaCl inhibited the methanogenic process and enhanced the hydrolysis and acidification, but with a high content of salt, acidification, and methanogenic were inhibited. Finally, Chen et al. [81] using batch assays analyzed different parameters that have impact in WAS-FW co-fermentation. pH, temperature and the mixture ratio were studied and concluded that the optimal conditions were pH 8, 37 °C and WAS/FW ratio of 40/60 in TS basis.

Table 2. Co-fermentation batch assays between WAS and FW

Type FW	**Mixture (WAS and FW)** [a]	**Inoculum**	**pH control**	**T (°C)**	**[VFA] (mgCOD·L^{-1})**	**Yield $_{\mathrm{VFA}}$ (mgCOD·gVS^{-1})**	**Ref.**
FW	2:1 (% wt)	N	Y (4)	20	2,426	n.r.	[40]
			Y (5)	20	n.r.	n.r.	
			Y (6)	20	5,023	n.r.	
			Y (7)	20	6,541	n.r.	
			Y (8)	20	8,237	n.r.	
			Y (9)	20	7,912	n.r.	
			Y (10)	20	n.r.	n.r.	
			Y (11)	20	4,273	n.r.	
			N	20	1,466	n.r.	
Potato peel waste	3:1 (VS) [1]	Y (UASB sludge)	Y (10)	35	n.r.	152	[39]
Potato peel waste	1:1 (VS) [1]		Y (10)	35	n.r.	268	
Potato peel waste	1:3 (VS) [1]		Y (10)	35	n.r.	344	
Synthetic FW (Fat, rice, meat and vegetables)	3:1 (VS) [1]		Y (10)	35	n.r.	140	
	1:1 (VS) [1]		Y (10)	35	n.r.	218	
	1:3 (VS)[1]		Y (10)	35	n.r.	282	
Rice	400:50 (mL)	Y (Seed sludge)	N	35	3,584	n.r.	[76]
Wheat	400:50 (mL)		N	35	4,447	n.r.	
Corn	400:50 (mL)		N	35	4,295	n.r.	
Bean	400:50 (mL)		N	35	n.r.	n.r.	
Chicken meat	400:50 (mL)		N	35	n.r.	n.r.	
FW	65-70/30-35 (%V) [2]	N	N	55	470	n.r.	[77]
			Y (7)	55	12,500	n.r.	
			Y (9)	55	24,500	n.r.	
			N	37	18,600	290-490	
			Y (7)	37	27,500		
			Y (9)	37	30,000		
			N	37	22,500	260-570	

[1] WAS: heat and alkali to accelerate hydrolysis: 105 °C for 3 hours and adjusted pH to 12.
[2] OFMSW: thermal pre-treatment, 76 h at 72 °C
[a] mixture of substrates basis units in brackets.
n.r. no reported

Continuation Table 2. Co-fermentation batch assays between WAS and FW

Type FW	Mixture (WAS and FW) a	Inoculum	pH control	T (ºC)	[VFA] (mgCOD·L^{-1})	Yield $_{VFA}$ (mgCOD·gVS^{-1})	Ref.
Rice	10 % FW (dry)	N	N	35	643	n.r.	[78]
	10 % FW (dry)		Y (3)	35	1,238	n.r.	
	10 % FW (dry)		Y (4)	35	1,510	n.r.	
	10 % FW (dry)		Y (5)	35	1,415	n.r.	
	20 % FW (dry)		N	35	2,509	n.r.	
	20 % FW (dry)		Y (3)	35	2,930	n.r.	
	20 % FW (dry)		Y (4)	35	1,589	n.r.	
	20 % FW (dry)		Y (5)	35	4,315	n.r.	
	30 % FW (dry)		N	35	4,944	n.r.	
	30 % FW (dry)		Y (3)	35	2,235	n.r.	
	30 % FW (dry)		Y (4)	35	6,012	n.r.	
	30 % FW (dry)		Y (5)	35	7,738	n.r.	
Rice	20 (C/N) [3]	Y (Seed sludge)	Y (4)	21	n.r.	n.r.	[79]
			Y (5)	21	n.r.	n.r.	
			Y (6)	21	n.r.	n.r.	
			Y (7)	21	n.r.	n.r.	
			Y (8)	21	n.r.	n.r.	
			Y (9)	21	n.r.	n.r.	
			Y (10)	21	n.r.	n.r.	
			Y (11)	21	n.r.	n.r.	
FW	0.24:1 (g)	N	Y (8)	20	2,800	n.r.	[59]
	0.24:1 (g)		Y (8)	35	n.r.	n.r.	
	0.24:1 (g)		Y (8)	50	2,300	n.r.	
	0.24:1 (g)		Y (8)	65	2,400	n.r.	

[3] Rice: first cooked for 0.5 h,1:2 (rice/tap-water in weight) and then cooled down to room temperature before being crushed by an electrical blender.

a mixture of substrates basis units in brackets.

n.r. no reported

Continuation Table 2. Co-fermentation batch assays between WAS and FW

Type FW	**Mixture (WAS and FW) a**	**Inoculum**	**pH control**	**T (ºC)**	**[VFA] (mgCOD·L^{-1})**	**Yield $_{\text{VFA}}$ (mgCOD·gVS^{-1})**	**Ref.**
FW	1:6 (VS)	N	N	20	n.r.	n.r.	[44]
			Y (6)	20	n.r.	n.r.	
			Y (7)	20	4,720*	n.r.	
			Y (8)	20	7,120*	n.r.	
			Y (9)	20	n.r.	n.r.	
			Y (10)	20	1,020*	n.r.	
	1:0.7 (VS)		Y (8)	20	6,110*	n.r.	
	1:2.5 (VS)		Y (8)	20	6,390*	n.r.	
	1:6.0 (VS)		Y (8)	20	7,120*	n.r.	
	1:3.5 (VS)		Y (8)	20	5,200*	n.r.	
	1:17.5 (VS)		Y (8)	20	2,810*	n.r.	
	1:6 (VS)		Y (8)	5	2,580*	n.r.	
	1:6 (VS)		Y (8)	35	2,810*	n.r.	
	1:6 (VS)		Y (8)	50	6,490*	n.r.	
Oleic acid	125:3 (wt)	n.r.	N	20	1,600	n.r.	[28]
	125:6 (wt)				1,937	n.r.	
	125:12 (wt)				2,871	n.r.	
FW +0.4 g/L NaCl	6 (gFW/gWAS) [4]	N	N	20	n.r.	n.r.	[80]
FW + 8 g/L NaCl			N	20	n.r.	639	
FW + 12 g/L NaCl			N	20	n.r.	n.r.	
FW + 16 g/L NaCl			N	20	n.r.	169	
FW	20 (C/N)	N	Y (4)	35	n.r.	391	[81]
			Y (12)	35	n.r.	140	
			Y (8)	5	n.r.	53	
			Y (8)	65	n.r.	318	
			Y (8)	35	n.r.	666	

[4] FW: was washed three times with tap water to eliminate the inherent NaCl
*COD in HPr
a mixture of substrates basis units in brackets.
n.r. no reported

3.3.2. WAS co-fermentation with agro-industrial waste

Agro-industrial (AI) waste is a type of organic waste that is gaining importance in co-fermentation. Mixing WAS with AI results in a more balanced ratio C/N, while WAS provides extra buffer capacity and provide a microbial community. Most papers focused on the C/N ratio to maximize the VFA production. There are a wide range of agro-industrial waste due to it is a seasonal waste, including corn straw, mushrooms and rice (Table 3).

The first author found to have studied agro-industrial waste co-fermentation was Guo et al. [38]. This article used different AI wastes as a co-substrate, corn straw and rice straw as straw group, and *Lentinus edodes* substrate an *Agaricus bisporus* substrates as a spent mushroom substrate. Straw group, which is rich in carbohydrate, has been shown that improve conversion of WAS proteins as there was a high proportion of propionic in the fermentation liquor. The SRT reduction increased the ORL and reduce the capital cost (smaller vessel). Additionally, SRT could affect the VFA production and profile because the microbe and their structures are closely related to the ORL. The optimal SRT was established at 8 days. Huang et al. [82], used henna plant biomass, which is rich in carbohydrates based on cellulose and lignocellulose. Using this co-substrate, the ratio C/N could be balanced. Also, the fact of providing the AI waste to WAS allowed the pH to be around 4-5. This pH permitted to carbohydrates and proteins to hydrolysed and inhibited the grow of methanogenic microorganisms. As for the VFA profile, the higher proportion of henna plant biomass, the less presence of acetic acid and propionic and more butyric and valeric acids. In a subsequent study, soy bean residue or tofu residue co-fermentation with WAS were studied by the same authors (Huang et al. [41]). This residue was rich in nutrients and its addition benefits the optimization of the fermentation medium, including the C/N ratio. If there was an excess of soybean residue, it can increase the content of lignocellulosic compounds that were more difficult to degrade and would make the process slower. The concentration of VFA was higher in the co-fermentation than the mono-fermentation. Besides, the higher proportion of tofu residue in the mixture, the higher the acetic concentration and the lower the propionic acid concentration in the VFA profile. Fang et al. [74], studied another type of cellulose-rich waste, *Oyster champost* (mushroom). Like soybean residue, due to its high cellulosic content makes the process slower. As other co-fermentation with AI, acetic was the predominant acid, followed by propionic and butyric acid due to the decrease of C/N ratio. Besides, more WAS added, less propionic but more butyric and valeric acids.

Other authors have researched the impact of other features on the co-fermentation performance, such as inoculum and pre-treatments. Xin et al. [83] studied the influence of inoculum (pig manure) in WAS-AI co-fermentation. The addition of the inoculum and the co-substrate (corn stank) permitted to adjust the C/N ratio. Following this, the VFA concentration was higher in this case. Regardless of whether inoculum was added or not in co-fermentation, the proportion of acid acetic descends while the propionic increased due to the pH and oxidation-reduction potential. Finally, Duan et al. [84], studied different pretreatment (ammonium hydroxide, sulfuric acid and thermal hydroxide) to soy sauce residue as co-substrate in co-fermentation with WAS. The pretreatment was based on decomposition of cellulose, hemicellulose and lignin. The best pre-treatment was ammonium hydroxide (21% NH_4OH) because contributed to change the pH useful for microorganism (started at 7 and decreased to 5.7) for hydrolysis and acidification steps and promoted a high decomposition of soy sauce lignocellulosic content.

Table 3. Co-fermentation experiments between WAS and agro-industrial waste

Type of AI	Mixture (WAS and AI) [a]	SRT (d)	Inoculum	pH control	T (ºC)	[VFA] (mgCOD·L^{-1})	Yield $_{VFA}$ (mgCOD·g[b]$^{-1}$)	Ref.
Corn straw	2:1 (VSS/VS) [1]	10	Y (seed sludge)	Y (10)	Mesophilic	8,743	416 (VS)	[38]
Rice straw		10				9,044	431 (VS)	
Lentinus Edodes		10				5,576	265 (VS)	
Agaricus Bisporus		10				5,880	280 (VS)	
Corn straw		8				9,943	473 (VS)	
Rice straw		8				10,492	500 (VS)	
Lentinus Edodes		8				627	299 (VS)	
Agaricus Bisporus		8				6,602	314 (VS)	
Corn straw		5				9,039	430 (VS)	
Rice straw		5				9,247	440 (VS)	
Lentinus Edodes		5				4,859	231 (VS)	
Agaricus Bisporus		5				4,899	233 (VS)	
Henna Plant Biomass	25 (% TS)[2]	n.r.	Y (WAS)	Y (8)	35	3,910	n.r.	[82]
	50 (%TS) [2]			Y (8)		5,326	n.r.	
	75 (% TS) [2]			Y (8)		7,875	n.r.	
Tofu residue	800 (mL WAS) + 0.16 (g VSS/g VSS WAS)	n.r.	n.r.	N	35	n.r.	n.r.	[41]
	800(mL WAS) + 0.32 (g VSS/g VSS WAS)					n.r.	n.r.	
	800 (mL WAS) + 0.48 (g VSS/g VSS WAS)					n.r.	n.r.	
	800 (mL WAS) + 0.64 (g VSS/g VSS WAS)					4,716	241 (VSS)	
	800 (mL WAS) + 0.8 (g VSS/g VSS WAS)					n.r.	n.r.	
	800 (mL WAS) + 0.96 (g VSS/g VSS WAS)					n.r.	n.r.	

[1] AI: thermal and alkaline pre-treatment.

[2] Henna plant biomasses was dried at 45 ºC for 8 h before usage

[a] mixture of substrates basis units in brackets.

[b] denominator of Yield units in brackets

n.r. no reported

Continuation Table 3. Co-fermentation experiments between WAS and agro-industrial waste

Type of AI	Mixture (WAS and AI) a	SRT (d)	Inoculum	pH control	T (ºC)	[VFA] (mgCOD·L^{-1})	Yield $_{VFA}$ (mg COD·g b^{-1})	Ref.
Oyster champost	75:25 (%TS) [3]	n.r.	Y (Mesophilic anaerobic digester)	N	30	n.r.	521 (VS)	[74]
	50:50 (%TS) [3]					n.r.	596 (VS)	
	25:75 (%TS) [3]					n.r.	505 (VS)	
Corn stalk	1:31 (L WAS/ g Corn Stalk) [4]	n.r.	Y (Pig manure)	n.r.	35	5,100	n.r.	[83]
	1:25.5 (L WAS/ g Corn Stalk) [4]		N	n.r.		4,800	n.r.	
Soy sauce residue	2:1 (VSS/VS) [5]	n.r.	n.r.	N	35	5,300	n.r.	[84]
	2:1 (VSS/VS) [6]					3,350	n.r.	
	2:1 (VSS/VS) [7]					4,517	n.r.	

[3] *Oyster champost* was dried at 70 ºC until constant weight.
[4] WAS: pre-treatment with commercial enzymes
[5] Soy sauce residues: Ammonium hydroxide pre-treated
[6] Soy sauce residues: Soy sauce residues: Sulfuric acid pre-treated
[7] Soy sauce residues: Thermal hydroxide pre-treated
a mixture of substrates basis units in brackets.
b denominator of Yield units in brackets
n.r. no reported

3.3.3. WAS co-fermentation with other substrates

There is a small part of the publications co-fermenting WAS with other co-substrates, such as PS and slaughterhouse waste (Table 4).

As for primary sludge as co-substrate, Maspolim et al. [85] studied the co-fermentation of WAS:PS at different pH. Maximum solubilization was obtained at a pH of 11. Acetic and propionic acids were the predominant VFA in all pH conditions except the no control (range of 5.8-6.3), 6 and 7. It was hypothesized that at neutral pH a fraction of the VFA were converted to methane. The optimal pH was 8, although no high solubilization was reached, the VFA yield was the highest obtained. Zurzolo et al. [86], also used PS as a co-substrate in order to recuperated phosphorus. The study was done with PS of two different WWTP. WAS proportioned to the co-fermentation enough alkalinity. As for mono-fermentation, PS produced more VFA than WAS. The combination of WAS-PS allowed for more dissolved phosphorus in the medium.

Liu et al. [87], studied glucose and bovine serum albumin as a model of slaughterhouse wastewater. During the co-fermentation of glucose, it was reported that in the 5 $g \cdot L^{-1}$ had the presence of butyric and valerate. It might be related to a change of metabolism pathways. Also, it was detected lactate, which it was used as electron donor to synthesis of butyrate and acetate. In case of the bovine serum albumin, the conversion to VFA was inefficient due to the high concentration of ammonium (1,000 $mgNH_4\text{-}N \cdot L^{-1}$ at day 8) during the degradation of co-substrate which inhibited the process. This fact was demonstrated by a batch test adding ammonia (from 0 to 1,000 $mgNH_4\text{-}N \cdot L^{-1}$) where both CO and H_2 were not consumed in the higher ammonium concentration indicating that the fermentation was inhibited.

Table 4. Batch and continuous assays of mixing WAS and other co-substrates

Co-substrate	**HRT (d)**	**SRT (d)**	**Mixture (WAS and Co-subs) a**	**Inoculum**	**pH control**	**T (ºC)**	**[VFA] (mgb·L^{-1})**	**Yield $_{VFA}$ (mgCOD·gVSS^{-1})**	**Ref.**
PS	n.r.	3	n.r.	Y(sludge)	N	35	n.r.	150	[85]
					Y (4)		n.r.	n.r.	
					Y (5)		n.r.	n.r.	
					Y (6)		n.r.	n.r.	
					Y (7)		n.r.	n.r.	
					Y (8)		n.r.	410	
					Y (9)		n.r.	n.r.	
					Y (10)		n.r.	n.r.	
					Y (11)		n.r.	n.r.	
					Y (12)		n.r.	n.r.	
PS_1	n.r.	n.r.	30:70 (VS)	n.r.	N	21	n.d.	n.r.	[86]
PS_2				n.r.			1,624 (COD)	139	
Glucose (Glc)	3	n.r.	120:5 (mL WAS /g Glc ·L^{-1})	n.r.	Y (9)	37	≈ 50 (mM)	n.r.	[87]
			120:15 (mL WAS /g Glc ·L^{-1})	n.r.			≈ 115 (mM)	n.r.	
Bovine serum albumin (BSA)			120:5 (mL WAS /g BSA ·L^{-1})	n.r.			≈ 80 (mM)	n.r.	
			120:15 (mL WAS /g BSA ·L^{-1})	n.r.			≈160 (mM)	n.r.	

a mixture of substrates basis units in brackets
b numerator of concentration of VFA units in brackets
n.r. no reported

3.4. Primary sludge as a main substrate

Primary sludge (PS) is the second most used main substrate in co-fermentation studies (Table 5). However, there is not a predominant co-substrate (Fig. 3). PS is characterized for being more biodegradable and for presenting higher soluble COD values than WAS (1.7-2.6 $gO_2 \cdot L^{-1}$ versus 0.2-0.3 $gO_2 \cdot L^{-1}$ respectively [28,40]).

Banerjee et al. [32] published the first paper using PS in co-fermentations, specifically the mixture between PS and potato industry wastewater. Banerjee et al. [32] studied different temperatures and HRT using above mixture. It could be seen that with an increasing of HRT there was a reduction in the rates due to a higher HRT resulted a longer contact. As for the temperature, there were a reduction of acidogenesis process in a mesophilic temperature (35 ºC) with a decreasing of VFA concentration. The authors hypothesized that this was because of the methanogenic archaea. As for the VFA profile the major acids were acetic acid and propionic acid.

Min et al. [88] studied the co-fermentation between PS and FW. Specifically, the author studied the influence of different parameters (i.e. HRT, FW proportion, pH and temperature) in PS-FW co-fermentation. The ambient temperature (18 ºC) attributed a major VFA concentration than mesophilic (35 ºC) because of methanogenic archaea. The VFA concentration was higher in the 10% wt. of FW when there was an increase of HRT. For the same reason mentioned above, higher HRT resulted in increased substrate degradation and more VFA production. As for pH, control this parameter did not enhance the VFA production.

Further other co-substrate is WAS reported by Ji et al. [31], which studied the effect of WAS addition and WAS with surfactant on co-fermentation. The addition of surfactant produced an increment of VFA concentration since its addition causes a greater solubilization of compounds such as proteins and carbohydrates. Besides, with the addition of the surfactant, more ammonia and phosphorus in the medium were found, in other words, there was an improvement on the solubilization.

Peces et al. [28], also studied the combinations between PS and oleic acid. There was a consumption of acetic acid during the co-fermentation due to other microorganism like sulphate-reducing bacteria or ordinary heterotrophs. However, this phenomenon did not always occur. Besides, the VFA production was higher with PS as the main substrate than WAS, mentioned in the *section 3.3.1*. Nicholson et al. [89] and Long et al. [90] studied the co-fermentation and co-digestion of PS and FOG at ambient temperature in a pilot scale. These studied showed that increasing a 20% of organic load through FOG addition increased the VFA concentration from 0.54 to 0.65 gCOD·L^{-1}.

Table 5. Primary sludge co-fermentation experiments

Co-substrate	HRT (d)	SRT (d)	Mixture (PS and Co-subs) [a]	Inoculum	pH control	T (ºC)	[VFA] (mg[b]·L^{-1})	Yield $_{VFA}$ (mgCOD·gVSS^{-1})	Ref.
FW	1	n.r.	10 (%wt FW)	N	Y (6.2-6.9)	18	150 (VFA)	n.r.	[88]
		n.r.	10 (%wt FW)		N (5.5-5.9)		270 (VFA)	n.r.	
		n.r.	25 (%wt FW)		Y (6.2-6.9)		2,155 (VFA)	n.r.	
		n.r.	25 (%wt FW)		N (5.5-5.9)		3,610 (VFA)	n.r.	
	3	n.r.	10 (%wt FW)		Y (6.2-6.9)		330 (VFA)	n.r.	
		n.r.	10 (%wt FW)		N (5.5-5.9)		1,190 (VFA)	n.r.	
		n.r.	25 (%wt FW)		Y (6.2-6.9)		450 (VFA)	n.r.	
		n.r.	25 (%wt FW)		N (5.5-5.9)		1,000 (VFA)	n.r.	
	5	n.r.	10 (%wt FW)		Y (6.2-6.9)		350 (VFA)	n.r.	
		n.r.	10 (%wt FW)		N (5.5-5.9)		1,185 (VFA)	n.r.	
		n.r.	25 (%wt FW)		Y (6.2-6.9)		55 (VFA)	n.r.	
		n.r.	25 (%wt FW)		N (5.5-5.9)		255 (VFA)	n.r.	
	1	n.r.	10 (%wt FW)		Y (6.2-6.9)	35	255 (VFA)	n.r.	
		n.r.	10 (%wt FW)		N (5.5-5.9)		680 (VFA)	n.r.	
		n.r.	25 (%wt FW)		Y (6.2-6.9)		515 (VFA)	n.r.	
		n.r.	25 (%wt FW)		N (5.5-5.9)		2,520 (VFA)	n.r.	
	3	n.r.	10 (%wt FW)		Y (6.2-6.9)		350 (VFA)	n.r.	
		n.r.	10 (%wt FW)		N (5.5-5.9)		405 (VFA)	n.r.	
		n.r.	25 (%wt FW)		Y (6.2-6.9)		220 (VFA)	n.r.	
		n.r.	25 (%wt FW)		N (5.5-5.9)		360 (VFA)	n.r.	
	5	n.r.	10 (%wt FW)		Y (6.2-6.9)		280 (VFA)	n.r.	
		n.r.	10 (%wt FW)		N (5.5-5.9)		310 (VFA)	n.r.	
		n.r.	25 (%wt FW)		Y (6.2-6.9)		35 (VFA)	n.r.	
		n.r.	25 (%wt FW)		N (5.5-5.9)		160 (VFA)	n.r.	

[a] mixture of substrates basis units in brackets

[b] numerator of concentration of VFA units in brackets

n.r. no reported

Continuation Table 5. Primary sludge co-fermentation experiments

Co-substrate	**HRT (d)**	**SRT (d)**	**Mixture (PS and Co-subs) a**	**Inoculum**	**pH control**	**T (ºC)**	**[VFA] (mgb·L^{-1})**	**Yield $_{VFA}$ (mgCOD·gVSS^{-1})**	**Ref.**
Oleic acid	n.r.	n.r.	125:3 (g)	N	N	20	2680 (COD)	n.r.	[28]
			125:6 (g)				4516 (COD)	n.r.	
			125:12 (g)				4155 (COD)	n.r.	
Wastewater potato industry	18	n.r.	50 (% V)	N	N	22	n.r.	n.r.	[56]
	18	n.r.				22	514 (COD)	n.r.	
	30	n.r.				22	627 (COD)	n.r.	
	30	n.r.				30	713 (COD)	n.r.	
	30	n.r.				35	419 (COD)	n.r.	
WAS	n.r.	6	1:1 (VSS)	N	N	21	n.r.	118	[31]
WAS+ biosurfactant	n.r.	6	1:1 (VSS) + 0.02 g TSS	N			n.r.	173	

a mixture of substrates basis units in brackets
b numerator of concentration of VFA units in brackets
n.r. no reported

3.5.Other main substrates

WAS and PS were not only the substrates utilised as main substrate. There are other main substrates but to lesser extent such as mixed sewage sludge (SS) [55], pig manure (PM) [47], *Sophora flavescens* [91], and food waste (FW) [92] (Table 6).

Rughoonundun et al. [55], focused on the C/N ratio through different mixtures between mixed sludge and sugarcane bagasse co-fermentation under neutral conditions of pH at 55 ºC. The mixtures had a VFA concentration than mono-fermentation (mix sludge only and bagasse only). The ratio of carbon-nitrogen increased as the proportion of bagasse in the mixture increases. The maxim VFA yield were obtained when the C/N ratio (weight basis) was between 13.2-24.5) whereas, when the C/N ratio was greater than 25, the yield decrease. This was attributed to a lack of nitrogen, which could had affected microorganism's activity. In all mixtures and blanks, the acetic acid was the predominant followed by butyric and then, propionic acid, except for MS only, that was caproic acid. This means, that C/N ratio could change the VFA distribution by (i) changing the metabolic pathways or/and (ii) had influence in the selection on microorganism. Then, Saritpongteeraka et al. [47], co-fermented PM with palm oil fresh fruit bunch using a cow manure as inoculum to improve hydrolysis, in order to optimize VFA generations from the percentage of pig manure and the flushing interval of the leach bed reactor. The pig manure had higher alkalinity that could maintained the pH level because of the high content of ammonia. With no control of the pH, this fell to an approximate value of 6. As other authors, acetic was the predominant acid, but with the addition of the main substrate, the production of valeric and hexanoic acid increase, due to the high content of proteins in pig manure. Besides, the addition of PM promoted the degradation of lignocellulosic compounds of bagasse. At last, the flushing interval did not show an impact in VFA production but has influence in VFA distribution because a longer flushing interval caused the appearance of butyric. Another author has focused on the term of agro-industry as a main substrate. Zheng et al. [91], was focus on different mixtures of crop (*Sophora flavescens*) and food waste with no pH control at 37 ºC using a pure culture (*Propionibacterium acidipropionici*). As it shows in Table 6, there was not a value for VFA, because of they were focus on L-lactic buy they explain the VFA profile. The lactic values were higher in co-fermentation than mono-fermentation. Lactic were the main product obtained, but in case of mono-fermentation of FW ethanol was a remarkable product. The best ratio for L-Lactic production was 1:1.5, due to the high concentration and conversion rate. Capson-Tojo, et al. [92], co-fermented FW with cardboard using inoculum, with no pH control at 35 ºC in order to optimized VFA and hydrogen production. The lower values of VFA yield were related to higher proportion of cardboard associated to a high content of TS. The main VFA was acetic followed by butyric. pH remains between 6.3-6.6 due to the buffer capacity of cardboard. Adding cardboard promoted a low yield of hydrogen, that transformed into an improvement in caproic yield which is produced by the acetic and hydrogen consumption.

Table 6. Other main substrates co-fermentation

Main Substrate	**Co-substrate**	**Flooding interval (FI) (h)**	**Mixture (Main and Co-subs) a**	**Inoculum**	**pH control**	**T (ºC)**	**[VFA] (mgb·L^{-1})**	**Yield $_{VFA}$ (mgb·gc^{-1})**	**Ref.**
MS	Raw bagasse[1]	n.r.	20 (% wt MS)	Y (previous reactor)	Y (7)	55	13,360 (VFA)	300 (VFA) (VS $_{fed}$)	[55]
			30 (% wt MS)				15,110 (VFA)	360 (VFA) (VS $_{fed}$)	
			40 (% wt MS)				15,080 (VFA)	360 (VFA) (VS $_{fed}$)	
			60 (% wt MS)				14,230 (VFA)	360 (VFA) (VS $_{fed}$)	
			80 (% wt MS)				13,300(VFA)	350 (VFA) (VS $_{fed}$)	
Pig manure	Oil Palm fresh fruit bunch	12	50:50 (% TS)	Y (Cow manure)	n.r.	n.r.	n.r.	119 (VFA) (g $_{substrate\ dry}$)	[47]
			25:75 (% TS)				n.r.	134 (VFA) (g $_{substrate\ dry}$)	
		24	50:50 (% TS)				n.r.	122 (VFA) (g $_{substrate\ dry}$)	
			25:75 (% TS)				n.r.	144 (VFA) (g $_{substrate\ dry}$)	
		48	50:50 (% TS)				n.r.	130 (VFA) (g $_{substrate\ dry}$)	
			25:75 (% TS)				n.r.	152 (VFA) (g $_{substrate\ dry}$)	
Sophora flavescens [2]	FW	n.r.	1:05 (dry)	Y(n.r.)	N	37	n.r.	n.r.	[91]
		n.r.	1:1 (dry)				n.r.	n.r.	
		n.r.	1:1.5 (dry)				n.r.	n.r.	
		n.r.	1:2 (dry)				n.r.	n.r.	
FW	Cardboard (CB)	n.r.	FW + CB (25 % TS) *	Y (granular and UASB dry digested)	N	35	n.r.	622 (COD) (COD $_{bio}$)	[92]
			FW + CB (30 % TS) *				n.r.	647 (COD) (COD $_{bio}$)	
			FW + CB (35 % TS) *				n.r.	616 (COD) (COD $_{bio}$)	
			FW + CB (40 % TS) *				n.r.	565 (COD) (COD $_{bio}$)	
			(FW +CB + H_2O) (25 % TS) *				n.r.	698 (COD) (COD $_{bio}$)	
			(FW +CB + H_2O) (30 % TS) *				n.r.	675(COD) (COD $_{bio)}$	
			(FW +CB + H_2O (35 % TS) *				n.r.	603 (COD) (COD $_{bio}$)	

[1] Raw bagasse: was mixed $Ca(OH)_2$ and distilled water and pretreated at 50 ºC for 8 weeks
[2] *Spphora flavescens*: were pre-treated with 8 % NaOH at 20 ºC for 24 h.
*Consider the inoculum in the mixing step

a mixture of substrates basis units in brackets.
b numerator of concentration of VFA and Yield units in brackets
c denominator of Yield units in brackets
n.r. no reported

4. FUTURE RESEARCH

Future research efforts on co-fermentation should not focus on controlling the pH of the mixture since it could change the VFA profile. In addition, by combining substrates, a mixture with enough buffer capacity can be obtained avoiding the addition of chemicals that involve an economic expense.

However, most of the studies have focused on macro-nutrients as C/N ratio. There is a wide range of parameters to consider. Further research is needed to understand the relative importance of factors controlling the VFA production such as substrates that provide positive aspects in terms of co-fermentation, optimal mixture, buffer capacity, microorganism, C/N ratio, temperature, pH, moisture, HRT and SRT. Finally, it is important to note that much of the experiments have been performed through batch assays. It would be advisable to perform more continuous co-fermentation experiments.

5. CONCLUSION

The co-fermentation process improves the yield and concentration of VFA. As for the main substrate most authors used WAS because it provided a community of microorganism, buffer capacity, moisture apart from its content of biodegradable compounds. Also, the most used mixture is the WAS between FW, followed by WAS and AI. Through the combination of WAS and FW it has been seen that the optimal pH was between 7-9. However, the proportion of both wastes has not been studied in detail yet. It has also been seen that the substrate has an important role in the VFA profile. Whereas, WAS and AI, there are a wide range of substrates for crops. Most of them were used in order to balance the C/N ratio. By means of these substrates it has been possible to see that an excess can cause a slowdown of the process. In reference to substrates, more relevance should be given to other potential substrates like FOGs or slaughterhouse wastes. Otherwise, one parameter to consider was the presence of ammonia in the fermentation liquid as it gives an idea of the fermentation process. Finally, most of the papers found refer to batch assays. In order to obtain more concise results, research should be done through continues assays and contemplate other important parameters like temperature, buffer capacity among others.

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