Chapter

Anaerobic Digestion of Organic Solid Waste: Challenges Derived from Changes in the Feedstock

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Abstract

Over the years, research on the anaerobic digestion of solid waste has mainly focused on single feedstocks with a fixed composition. Nevertheless, the impact assessment that drastic changes in the type and composition of feedstock might have on AD process stability has not been investigated in depth. The existence of a wide variety of organic solid waste whose generation and composition are highly dependent on seasonality, just as the possibility of using treatment plant facilities already in operation for treating new waste, makes it necessary to improve our knowledge of transitory states in AD. This chapter aims to provide insight into research on transitory states during the AD process when the type or composition of the feedstock has suffered a change to assess whether the AD process was finally able to adapt to system disturbances. Information about process stability control and microbial population adaptation, among others, derived from the transition states will be addressed.

Keywords: organic waste management, process stability, seasonality, substrate change, transient conditions

1. Introduction

High global population growth has led to an excessive increase in solid waste generation. According to the United Nations, at least 7000 and 10,000 million tons of solid waste are collected worldwide yearly [1]. The principal sectors responsible for this amount of solid waste are as follows: (1) construction and demolition (C&D) (34%); (2) municipal solid waste (MSW) (24%); (3) industrial (21%), and (4) commercial (11%) [1].

The MSW is the waste generated by households, mainly composed of an organic fraction, plastic, and paper. Although the organic fraction in MSW varies according to income levels countries (high-income countries ~20–40% and low-income countries ~50–70%), sociocultural patterns, and climatic factors, it could be considered that around half of the MSW would correspond to the organic fraction [2]. It means that about 840–1200 million tons of organic solid waste would be globally generated [1, 3]. It may even be argued that this amount of organic solid waste would be even

higher as it does not consider the agro-industrial sector, whose waste is composed primarily of organic matter. The producers hardly report data from this sector. However, a rough estimate by the International Solid Waste Association (ISWA) concluded that approximately 10,000–20,000 million tons of waste are generated annually by crops, farms, vineyards, dairies, and other agri-food industries [1]. In summary, it could be stated that the amount of organic solid waste generated globally is highly significant, and its leadership in the waste generation field remains a relevant issue.

Globally, in 2018, about 19% of solid wastes were recycled and/or composted, 11% incinerated, 37% disposed of in landfills (with or without gas collection), and 33% disposed of in open dumps (uncontrolled waste disposal) [3]. The high percentage of waste derived from landfilling or open dumps indicates that there are still great opportunities for improvement in the management of organic solid waste. Within the technologies available for recycling and composting, anaerobic digestion (AD) has been recognized as an effective and interesting waste management technology, since it can produce green energy when converting organic matter from waste into biogas [4–6].

Increasing solid waste generation requires cost-effective and environmentally friendly processes, such as AD. With proper control of the AD process, this technique could be adapted to different operating conditions and changes in the feedstock. It would allow the possibility of treating a greater quantity and variability of seasonal waste (e.g. MSW; fruit and vegetable waste (FVW); and juice company waste) in a single solid waste plant, using existing facilities [7]. This advantage could even improve the biogas production and the economic viability of the plants [7, 8]. Because of these reasons, the ability of the AD process to adapt to different changes could be a compelling topic. In this context, variations of parameters have been extensively studied, such as organic loading rate (OLR), hydraulic retention time (HRT), or the operational temperature in the AD process [9–17].

Despite the existing knowledge about the previously cited operational parameters, feedstock type or composition changes in the AD process have been poorly investigated and could affect the process behavior. When an AD process is carried out under fixed operating conditions, there is a steady state in which the system conditions remain constant. However, when a change or disturbance affects the system, e.g. a change in the feedstock type or composition, the existing stability conditions can be lost, resulting in a transitory state. A transitory state could be defined as the period that elapses from when the change is applied to the system until system stabilization is reached. During this period, parameters such as the biogas production and composition or the concentration of volatile fatty acids (VFAs) could change because of biodegradability, pH, organic matter content, and other feedstocks' characteristics. This could directly affect the subsequent AD performance [7].

It has also been reported that the way how changes in conditions are made can cause different results in the system's adaptation [17]. For example, an aggressive change usually results in considerable instability in the system. On the contrary, a gradual change usually entails fewer fluctuations, because the system has more time to adapt to the new conditions. For this reason, the evaluation of the change influence on the development of the transitory state is a fundamental step to realize in the AD process [9].

This chapter aims to provide an insight into the available research on transitory states during the anaerobic digestion process when the feedstock type or composition has suffered a change, to assess the adaptation of the anaerobic digestion process to the system perturbations.

2. Monitorization of the anaerobic digestion process stability of organic solid waste during transitory states

This section describes the control and operational parameters relevant for determining the stability and the microbial population adaptation of AD processes. These factors have been reported as fundamental in the literature for transitory states during the AD processes, when the feedstock type or composition changes.

2.1 pH, alkalinity, and volatile fatty acids concentration

Monitoring parameters such as pH, alkalinity, and VFAs concentration are fundamental indicators of the equilibrium and stability of the AD process [6, 18].

Microbial growth and activity strongly depend on the environmental pH [19–21]. According to literature, methane producers are most active at a neutral pH, i.e. between 6.5 and 8.5 [22, 23], while at lower pH (5.0–6.0), its activity decreases severely, being active only for the acids-producing microorganisms [21]. If pH is rapidly increased or decreased concerning the existing environmental conditions, the microbial activities of specific microbial species could be inhibited. Notably, the methanogenic archaea inhibition would affect the activity of anaerobic microorganisms and, subsequently, the whole AD process performance [24]. The rapid increase or decrease of pH values could mostly occur for substrates from different origins whose physicochemical characteristics are not similar [4]. Arhoun et al. [23] reported different pH buffering processes that, while remaining active, can hide possible instabilities. Still, when the buffering capacity is depleted in the long term, abrupt pH changes could cause severe problems to the digester operation. However, other parameters can be an early warning for pH buffer depletion. Among the most used are total (TA), partial (PA), or intermediate (IA) alkalinity.

Alkalinity could be defined as the ability of the AD liquor of the mixture to buffer the possible generation of acids produced during the biological process and, hence, mitigate potential pH changes [7]. The alkalinity in the AD liquor mixture is mainly provided by the non-protonated forms of VFAs and the carbonate system. If no other species interfere within the pH range of anaerobic digesters, a VFAs accumulation would be directly related to the breakdown of the bicarbonate buffering capacity [25]. The PA, IA, and TA measurements can evaluate the relative buffering substances concentrations. TA measures the combined effect of different buffer systems and is calculated as TA = PA + IA. PA corresponds to the buffer capacity of the carbonate system, just as ammonium/ammonia. In contrast, IA is the difference between TA and PA and corresponds to the buffer capacity of the non-protonated forms of VFAs. Alkalinity titrated down to 5.75 pH value is defined as a PA, whereas TA is titrated to 4.30 [20, 26]. Some authors have reported 2000–4000 mg of $CaCO_3$ L^{-1} PA values as typical for properly performing digesters under mesophilic conditions and feeding organic solid waste [11, 27, 28]. However, in terms of stability, the evolution of parameters over time is more important than the actual concentration, acting as an early warning [23].

Several studies also reported alkalinity ratios as monitoring parameters used as early warning tools [23, 25, 29]. The process stability can be evaluated by the IA/PA ratio, which involves the acid concentration in the system (IA), and the buffer capacity provided by the carbonate species (PA). If the PA is insufficient to buffer the IA, the digester will be acidified, and the activity of microorganisms, especially methanogens, will be inhibited [29]. Therefore, to consider the process stable, the IA/PA ratio

must be kept below 0.4. Some authors also indicate IA/TA ratio as a parameter for monitoring the anaerobic digestion process. However, this has lower sensitivity than the IA/PA ratio [25].

The VFAs are produced during the anaerobic degradation of organic solid waste, and their evolution provides information about the performance of the different AD steps [7, 15, 30]. Especially, the substrates with high biodegradabilities, such as fruit, vegetable, or food waste, have a higher tendency to generate VFAs. The most common VFAs are acetic (C2), propionic (C3), butyric (C4), and valeric (C5) [8]. Acetic acid has been described as the least toxic fatty acid. On the contrary, propionic acid concentration has been associated with system failure, being even more inhibitory than butyric acid [18, 31]. The propionic acid accumulation is probably related to its conversion being the least thermodynamically favorable [6]. According to some studies, a propionic acid concentration in the range of 0.45–3.00 g COD L^{-1} (COD, chemical oxygen demand) has a high potential to inhibit the process. Obviously, inhibition will also depend on the substrate treated and the operating conditions [6, 32, 33]. As a result, propionic acid is usually presented as the main parameter to follow when analyzing the stability of AD [7].

Another commonly used parameter to monitor the stability of the anaerobic digestion process is the ratio of volatile fatty acids (VFAs) to total alkalinity (TA) (VFA/TA or FOS/TAC ratio) [4, 34]. This parameter is related to the buffering capacity, represented by the total alkalinity, for a given effect of the VFA on the pH of the AD liquor mixture [23]. According to the literature, there are three critical VFA/TA ratio values. If VFA/TA ratio is lower than 0.40, the digester should be stable. When the ratio ranged from 0.40 to 0.80, the digester performance would present some signs of instability, while the VFA/TA ratio higher than 0.80 indicates significant instability in the digester [7, 11, 35].

2.2 Specific energy loading rate (SELR)

The specific energy loading rate (SELR) is, according to the literature, one of the parameters for evaluating the AD process stability, since it is useful for determining allowable organic loading rates. The SELR is defined as the quotient between the daily feed organic load (expressed in g of tCOD $(L{\cdot}d)^{-1}$) and the active biomass inside the digester (expressed in g VSS L^{-1}) (VSS, volatile suspended solids) (Eq. (1)) and can be considered as an indicator of food to mass ratio (F/M) [19]. Thus, if the food mass in the feedstock exceeds the mass of decomposer microorganisms, it could cause a metabolic imbalance because of the acidification and inhibition of methanogenic microorganisms [36]. On the contrary, if the abundance of food available is insufficient, the metabolism of the microorganisms could be affected [37]:

$$\text{SELR} = \frac{Q{\cdot}[tCOD]_{inlet}}{[VSS]{\cdot}V_{working}} \tag{1}$$

where Q is the inlet flow rate (L d^{-1}), $[tCOD]$ is feeding total COD concentration (g L^{-1}), $[VSS]$ is digestate volatile suspended solids concentrations (g L^{-1}), and $V_{working}$ is the working volume of the digester (L).

According to Azevedo et al. [38], the limit value for SELR is 0.4 d^{-1}. A higher value indicates a potential instability between the biomass of the microbial consortium and the loading of the feed mixture.

2.3 Removal of organic matter (volatile solids and COD)

Methane production should be stable if there is no accumulation of organic compounds inside the digester. A feeding of substrates with poor biodegradability could increase the content of volatile solids inside the reactor. Likewise, if the feed rate exceeds the rate of degradation by the microorganisms, organic compounds will accumulate inside the reactor, and methane production will be impacted. It is worth noting that the biodegradability capacity of a digester would depend on substrate characteristics and microbial degradation capacity. Therefore, this variable would be useful to evaluate the adaptation of an AD reactor to new substrates by comparing the biodegradability values in the digester with the expected for the added substrates. In that sense, biomethane potential tests can be a powerful tool to provide a reference framework for the biodegradability of the substrates [39]. The volatile solid removal is determined by Eq. (2) [23, 35]:

$$VS_{\text{removal}} = \frac{VS_{inlet} \cdot VS_{digestate}}{VS_{inlet}} \tag{2}$$

2.4 Total ammonia nitrogen (TAN)

Feedstocks with high content of proteins, i.e. with high content of nitrogen compounds, could induce high total ammonia nitrogen (TAN) in the AD process leading to biomass inhibition [6, 35]. A C/N ratio of 10–30 in the feedstock has been reported in the literature, which could avoid ammonium inhibition [20, 23]. Ammonia inhibition usually leads to a decrease in methane production rate and an increase in intermediate VFAs. Ammonia levels in the 200–1000 mg NH_4-N L^{-1} have no adverse effect, while inhibition occurs between 1500 and 3000 mg NH_4-N L^{-1}, especially at higher pH values, and complete inhibition, at all pH values, above 3000 mg NH_4- N L^{-1} [35].

2.5 Biogas production and composition

Biogas production is a crucial measure of the AD process status. If at a given OLR, there is a decrease in biogas production or biogas production rate that does not correspond to the degradation of the fed load, it could be considered a warning sign that the process is not working at its optimum [20]. According to the literature, the production of biogas or methane can be expressed as gas production rate (GPR), specific gas production (SGP), specific methane production (SMP), or specific methane yield (SMY), among others [6, 38, 40]. The GPR is expressed as the biogas volume generated per day to the reactor volume. The SGP and SMP/SMY are the biogas or methane volume generated by the mass of volatile solids feeding [6]. Generally, specific parameters are used to compare the stability of anaerobic digestion processes developed at different OLR values.

The organic matter degradation by microorganisms produces different types of gasses contained in the biogas. The biogas composition is mainly methane (45–85%), carbon dioxide (15–45%), and other gases such as hydrogen sulfide, ammonia, and nitrogen. The accurate proportion of gasses in the biogas depends on the process conditions and the feedstock [20].

A change in the microbial community could generate a different biogas composition. If there is an accumulation of hydrogen in the process, the hydrogenotrophic

methanogenic microorganisms could be inhibited. On the contrary, if acetic acid accumulates, the acetoclastic methanogenic microorganisms could be inhibited. In both situations, methane production could be affected [18]. Also, the different compositions of the feedstock can directly affect biogas generation. Alibardi & Cossu [41] found that a higher proportion of carbohydrates in the substrate results in a more significant biogas generation. Not so when the substrate is mainly composed of lipids or proteins.

2.6 Microbial population adaptation

Commonly, the AD process involves several stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [20, 21]. First, hydrolytic microorganisms are responsible for breaking down complex organic matter, such as proteins and carbohydrates, into simpler compounds. In the acidogenesis stage, the simpler compounds are biodegraded by acidogenic into VFAs, alcohols, H_2, and CO_2. Then, acetogenic microorganisms transform them into acetic acid, H_2, and CO_2. Finally, methanogens convert these products into CH_4 and CO_2 [42] following two pathways. One is carried out by acetoclastic methanogens, which can convert acetate into CH_4 and CO_2; the other is performed by hydrogenotrophic methanogens, which convert H_2/CO_2 to CH_4 [43]. These relationships play a crucial role in the anaerobic process leading to a balance between populations. For example, hydrogenotrophic methanogens are responsible for maintaining a low partial pressure of H_2 (<10 Pa), which is necessary for the functioning of the intermediate trophic group [44]. Therefore, the AD process stages efficiency is closely associated with the abundance and activities of specific anaerobic microbial communities. Many studies have reported that the diversity and abundance of microbial communities are closely associated with the digestion conditions, such as OLR, pH, temperature, HRT, and types of digestion substrates [2, 21], and an active anaerobic microbial communities imbalance could reduce the efficiency of the AD process [21, 45].

In anaerobic digesters, the stability of the microbial population and the relationships between groups (i.e. acetate utilizing methanogens/hydrogen utilizing methanogens ratio, and sulfate-reducing bacteria/methanogens ratio) are widely used parameters to establish the stability of the digesters. However, there is a lack of studies reporting the effect of feedstock's type and composition on community structure and microbial activity changes. Zahedi et al. [42] have observed that although the number of microorganisms is essential in many microbial ecology studies of anaerobic digestion, operating with actual and changing wastes under realistic circumstances, MSW could be not a key parameter to control the process. It was concluded that stability and good microbial community dynamics (flexibility to adapt in response to changes in environments, particularly to changes in the substrate and operating conditions) are essential factors for the stable performance of the reactors [42].

So, although some researchers have documented that feedstock composition and OLR may influence bacterial and archaeal communities, there is a lack of consensus on the impact assessment that drastic changes in the type and composition of feedstock might have on community structure changes, bacterial density increases, and microbial diversity. Furthermore, nowadays, the complexity, cost, and high expertise required for this kind of analysis advocate for using the microbial analyses as a supplementary tool for gaining deep knowledge of the reactors, but not for the routine monitoring of the AD process.

3. Feedstock changes in the anaerobic digestion process of organic solid waste

This section reports information compiled from the literature on studies investigating the transitory states during the AD process when the type or composition of the feedstock fed has changed. A change in feedstock type would refer to a feed with different substrates, whereas a change in feedstock composition would refer to a feed where the

Feedstock	Feeding	Type of AD	Monitoring parameters	Temp.	OLR	Methane production	Ref.
Cow manure (CW), Sugar beet pulp (SBP), Linen (Ln), and Wheat straw (WS)	CW/Ln/WS CW/Ln/SBM	AcoD	Biogas production Biogas composition % VS removal	Mesophilic (37 ± 1°C)	1.0 g VS $(L{\cdot}d)^{-1}$	CW/Ln/WS: 0.064 L g VS^{-1} CW/Ln/SBM: 0.191 L g VS^{-1}	[46]
Fruit pulp waste by a fruit juice company	Peach Raspberry White guava	AmD (Two steps)	VFAs Biogas production Biogas composition Microbiology %COD removal	Mesophilic (30°C)	7.0 to 25.7 g COD $(L{\cdot}d)^{-1}$ (acidogenic reactor) 1.9 to 7.4 g COD $(L{\cdot}d)^{-1}$ (methanogenic reactor)	Peach: 0.30 L g COD^{-1} Raspberry-I: 0.30 L g COD^{-1} Raspberry-II: 0.32 L g COD^{-1} White guava: 0.37 L g COD^{-1}	[47]
Municipal sewage sludge (SS) and orange peel (OP) from a bar	Stage I: SS Stage II: SS+OP pre-reated (OL) Stage III: SS+Sieved OL (SOL)	AmD and AcoD	pH Biogas production Biogas composition SELR	Mesophilic (37 ± 2°C)	1.80 ± 0.31 g VS $(L{\cdot}d)^{-1}$	SS: 0.100 L g VS^{-1} SS+OL: 0.177 L g VS^{-1} SS+SOL: 0.301 L g VS^{-1}	[40]
Fruit pulp waste by a juice-producing company	Peach Apple	AmD (Two steps / lab scale)	VFAs Biogas production Biogas composition Microbiology %COD removal	Mesophilic (30–37°C)	21.2 to 51.1 g COD $(L{\cdot}d)^{-1}$ (acidogenic reactor) 0.2 to 12.2 g COD $(L{\cdot}d)^{-1}$ (methanogenic reactor)	Peach: 0.25 L g COD^{-1} Apple: 0.31 L g COD^{-1}	[45]
	Pear Apple	AmD (Two steps / pilot scale)		Mesophilic (30°C)	12.2 to 22.2 g COD $(L{\cdot}d)^{-1}$ (acidogenic reactor) 1.8 to 3.3 g COD $(L{\cdot}d)^{-1}$ (methanogenic reactor)	Pear: 0.30 L g COD^{-1} Apple: 0.30 L g COD^{-1}	

Feedstock	Feeding	Type of AD	Monitoring parameters	Temp.	OLR	Methane production	Ref.
Artificial organic fraction (AOF) and four agro-industrial wastes (AWs) (cotton gin waste (CGW), winery waste (WW), olive pomace (OP), and juice industry waste (JW)) *Assays A: single feedstock Assays B: co-feedstock in a ratio of 40:60 in VS AW: AOF	A-I: CGW-WW-OP-JW-CGW A-II: WW-OP-JW-CGW-WW A-III: OP-JW-CGW-WW-OP A-IV: JW-CGW-WW-OP-JW	AmD	pH Alkalinity VFAs VFA/TA ratio TAN Biogas production	Mesophilic (35°C)	1.0 g VS $(L{\cdot}d)^{-1}$	A-I: 0.201 → 0.148 L g VS^{-1} A-II: 0.297 → 0.338 L g VS^{-1} A-III: 0.117 → 0.151 L g VS^{-1} A-IV: 0.335 → 0.369 L g VS^{-1}	[6]
	B-I: CGW-WW-OP-JW-CGW B-II: WW-OP-JW-CGW-WW B-III: OP-JW-CGW-WW-OP B-IV: JW-CGW-WW-OP-JW	AcoD				B-I: 0.280 → 0.284 L g VS^{-1} B-II: 0.384 → 0.405 L g VS^{-1} B-III: 0.268→ 0.319 L g VS^{-1} B-IV: 0.402 → 0.429 L g VS^{-1}	
Sewage sludge (SS) from a municipal WWTP and fruit waste from a fruit processing industry (peach waste (PW), banana waste (BW), and apple waste (AW))	Stage I: SS Stage II: SS + PW Stage III: SS + BW Stage IV: SS + AW Stage V: SS	AmD and AcoD	Alkalinity VFAs VFA/TA ratio Biogas production Biogas composition	Mesophilic (37 ± 2°C)	1.2 g VS $(L{\cdot}d)^{-1}$ (AmD) 3.0 g VS $(L{\cdot}d)^{-1}$ (AcoD)	SS: 0.28 L g VS^{-1} SS+PW: 0.20 L g VS^{-1} SS+BW: 0.30 L g VS^{-1} SS+AW: 0.26 L g VS^{-1} SS: 0.28 L g VS^{-1}	[7]

AmD: anaerobic mono-digestion; AcoD: anaerobic co-digestion, where OLR, organic loading rate; VFA, volatile fatty acids; TA, total alkalinity; VS, volatile solids; TAN, total ammonia nitrogen; SELR, specific energy loading rate; and COD, chemical oxygen demand.

Table 1.
Type of feedstock change.

percentage composition of the various substrates that compose the feedstock varies. In these works, to evaluate whether the AD process was finally able to adapt to the perturbations of the system, the parameters previously described in section 2 were assessed.

3.1 Type of feedstock

This section includes all the studies found in the literature that evaluate the AD process stability when changing the feedstock type (**Table 1**). These studies mostly use seasonal wastes generated in agri-food industries, i.e. fruit or vegetable processing, as feedstock, such as fruit pulp waste by a juice-producing company, winery waste, olive pomace, or sugar beet pulp. All these studies agree that the seasonality of the fruit and vegetable processing industries and waste from different crops would complicate operating a digester under the same conditions over a long period, because the waste supply could be changed or discontinued frequently [7, 46]. Therefore, using a single digester, fed with multiple feedstocks generated in the same geographical area and strongly dependent on seasonality, would require a deep knowledge of the behavior of the AD process when exposed to the resulting feed changes.

Despite the limited literature on the field, there is a wide variety of approaches for assessing the effect of feedstock type change on the stability of the AD process. Feedstock type change has been evaluated in mono-digestion processes with sequential feeding [6] or two-stage processes [45, 47]. It has also been studied in the transition from mono-digestion to co-digestion by applying feedstock change in the latter case [7, 40] and co-digestion processes with sequential feeding [6] or multi-substrate [46].

All research that has monitored pH as a stability parameter has used substrates from similar origins and characteristics, so it has reported stable pH values between 7.0 and 8.0 [6, 40]. As for monitoring alkalinity, VFAs concentration, and VFA/TA ratio, variable results have been reported, all of them related to the varying composition of the feedstocks fed. According to Pellera et al. [6], who evaluated the sequential feeding of four agro-industrial feedstocks (CGW → WW → OP → JW; cotton gin was (CGW), juice industry waste (JW), olive pomace (OP), and winery waste (WW)), the VFAs concentration was higher during the first two stages, especially for the experiments that started with feeding the most biodegradable feedstocks, i.e. WW and JW. Then, the values decreased to stable levels until the end of the experimentation, while the TA showed an increasing trend. Similarly, the VFA/TA ratio followed the same trend that VFA, without exceeding the value of 0.4, thus corroborating the system's stability. In contrast, Fonoll et al. [7] stated that feedstock changes did not increase VFAs concentration. However, due to the different biodegradability of fruit wastes, methane production and digester alkalinity changed to a lesser extent. The VFA/TA ratio values showed stability while changing feedstock despite the observed alkalinity fluctuations. Carvalheira et al. [45] evaluated the feedstock change in a two-stage anaerobic mono-digestion process, using fruit pulp waste by a juice-producing company as a substrate. During the monitoring of the acidogenic reactor, differences in the profile of fermentation products, i.e. VFAs, lactic acid, and ethanol, were identified and quantified when using other fruit pulp wastes. These results were attributed to carbohydrate concentration and OLR on the effluent composition. On the contrary, Mateus et al. [47], who also evaluated the feedstock change in a two-stage anaerobic mono-digestion process, reported a stable fermentation product profile, regardless of the different carbohydrate concentrations in the substrates and OLR changes. The difference between both studies could be attributed to the fact that the OLR range used by Carvalheira et al. [45] to apply the feedstock change was higher.

The evaluation of AD process stability when feedstock type changes through SELR was reported by Carvalho et al. [40]. The SELR values ranged between 0.22 and 0.33 d^{-1}, without significant differences, keeping the values below 0.4 d^{-1} and ensuring that the digestor worked under stable conditions (section 2.2).

Concerning methane production and composition, available research reports stable production values and relates their differences to the characteristics and biodegradability of the feedstocks fed [6, 7, 40, 46]. However, one of the most remarkable results dealing with methane production was reported in the study by Pellera et al. [6], which evaluated a sequential feeding by mono-digestion and co-digestion. Methane production with the same feedstock fed in different feeding sequences had similar values, attributed to an immediate response of the microbial population to each substrate. In fact, after providing the digesters with four feedstocks (mono- or co-substrate) in sequential order, the last feeding was carried out with the feedstock that had been fed first in each assay. The results demonstrated that the final methane production values were higher than their first values in all cases (**Table 1**). As an explanation for these results, they suggested a positive level of microbial population adaptation, albeit also possible presence of higher amounts of degradable material in the reactors as it was fed on 14 times. On the other hand, Carvalheira et al. [45] showed an increase in fermentation product concentration in the effluent of the methanogenic reactor, with the change of substrate reaching a maximum of 6.565 g COD L^{-1}, even after decreasing the OLR. There was a significant acetic and propionic acid accumulation, 2.44 and 1.44 g COD L^{-1}, respectively. The decrease in OLR and biodegradable matter accumulation decreased methane production when peach pulp was replaced by apple pulp, from 4.33 to 3.38 g COD $(L{\cdot}d)^{-1}$, respectively (**Table 1**). The decrease in process efficiency indicated that the microbial community was affected by the influent change and could not treat the apple influent with a high OLR as efficiently as the previous peach influent. Despite influent variations, stable performance of the methanogenic stage was achieved, probably due to the buffering capacity of the acidogenic community at the initial stage. In contrast, Mateus et al. [47] reported differences in the biogas composition generated in the acidogenic step in evaluating the two-stage mono-digestion process. In this case, the difference in carbohydrate concentration seemed to mainly affect the gas production and composition in the acidogenic reactor. No hydrogen production was detected with the peach pulp waste but with the raspberry and white guava pulps waste, ranging from 4 to 34%.

Reviewed studies state that whether or not there was instability during the whole AD experiment when the feedstock type changes, the microbial population has acclimatized well to the change. Different authors have argued that an acclimatization period would not be necessary with each change of feed material, as the microbial community is already adapted to substrates of a similar nature. Studies assessing changes in the microbial population ensure that the reactors were abundant in archaeal methanogens, mainly *Methanosaeta*, responsible for acetoclastic methanogenesis, the most common process in AD processes involving the production of CH_4 and CO_2 from acetate. *Methanobacterium*, microorganisms responsible for hydrogenotrophic methanogenesis involving methane production from CO_2 and H_2, were also identified. The microbial community composition remained relatively constant over time in each experiment [45, 47].

3.2 Composition of feedstock

This section includes all the studies found in the literature that evaluate the AD process stability when changing the feedstock composition in the influent (**Table 2**). These studies mostly use a mixture of wastes whose composition is strongly dependent on seasonality, such as food waste, fruit and vegetable waste from wholesale markets, meat waste, or the organic fraction of municipal solid waste (OFMSW).

All these studies aimed to evaluate changes in feedstock composition in the influent on the digesters' stability. However, some assays kept the organic loading rate (OLR) constant throughout the experimentation, despite the change in influent composition, to attribute the changes in reactor behavior to the change in composition [48, 49]. On the contrary, in other studies, by ignoring the intrinsic modification of the OLR due to the change in composition due to percentage (w:w or v:v) increase, they evaluated the combined effect of these two factors [2, 4, 23, 26, 34, 35, 43].

Unlike described in section 3.1, despite the limited literature in this field, not many different approaches have been studied to assess the effect of changing feedstock composition on the stability of the AD process. Feedstock composition change has been evaluated in the single- and two-stage mono-digestion process at the pilot scale [4, 34] and in the transition from mono-digestion to co-digestion by increasing the co-substrate percentage in the feed mixture [2, 23, 26, 35, 43, 48, 49]. Some studies have implemented changes in compositional percentages to improve methane production by adjusting the C/N ratio and the most optimal fruit and vegetable percentage.

Some research that monitored pH as a stability parameter by changing the feedstock composition in the influent has reported stable pH values between 7.0 and 8.0 and within the optimal range described in the literature for methanogenic bacteria [2, 26, 35, 43]. However, some other studies have reported fluctuations in these parameters. For example, Arhoun et al. [23], who evaluated the change in feedstock composition and seasonal variations, observed a very slight trend of decreasing pH with winter substrate. This slight acidification was related to the mixture's pH value, which was 3.5, lower than the other seasons, approximately 4.8. Masebinu et al. [4] and Scano et al. [34] have also reported a slight decrease in pH values with an increasing percentage of fruit in the feedstock composition, a higher percentage of citrus fruit, and fruits with a very high content of simple sugars, respectively. In addition, García-Peña et al. [49] have also described a quick drop in pH when feeding the reactors with FVW that was solved by adding buffer (NaOH 0.8 M) to supply the appropriate buffering capacity and avoid excessive pH drop under unbalanced conditions.

As indicated in section 3.1, regarding the monitoring of alkalinity, VFA concentration, and VFA/TA ratio, variable results have been reported, all of them related to the varying composition of the feedstocks fed and the specific stress situations performed during reactor feeding.

Some authors assessing alkalinity and VFAs and their corresponding ratios report stable values, and compliance with stability recommendations for VFA/TA and IA/PA ratios has been reported in the literature [23, 26, 35]. Tonanzi et al. [2] have reported a slight transient accumulation of acetic acid (60% of the soluble content) as a result of an increase in OLR up to 3.5 g VS $(L\ d)^{-1}$, reflected in a decrease in methane production. Propionic acid remained at low levels. The robustness of the microbiome and buffering capacities ensured quick recovery, acetic acid was eliminated, and methane production reached a stable value of 0.29 $NL^3\ CH^4\ g\ VS^{-1}$ (**Table 2**). Masebinu et al. [4] have observed two significant instabilities caused by reaching high OLRs (3.42 and 4.06 g VS $(L\ d)^{-1}$), i.e. mixtures with high fruit concentrations. A high OLR causes the system to be susceptible to fluctuations in feed composition and operating parameters. Maintaining a high fruit fraction in the substrate mixture caused a decrease in pH, an increase in the VFA/TA ratio above the stable region (0.45 and 0.53), and an eventual reduction in biogas production. As the percentages of fruit in the feed mix were reduced, all improved and returned to stability with improved biogas yield. Both pH and VFA/TA immediately indicated instability for an exceptionally high fruit concentration.

Feedstock	Feeding	Type of AD	Monitoring parameters	Temp.	OLR	Methane production	Ref.
Waste activated sludge (WAS) and food waste (FW) (FW:WAS ratio, on a VS basis)	Stage A-I: 100:0 Stage A-II: No feed Stage A-III: 100:0 Stage A-IV: No feed Stage A-V: 30:70	AmD and AcoD	pH VFAs Biogas production Microbiology	Mesophilic (37°C)	0.8–3.5 g VS $(L{\cdot}d)^{-1}$	A-V: 0.17 NL g VS^{-1}	[2]
	Stage B-I: 70:30 Stage B-II: 90:10 Stage B-III: 95:5 Stage B-IV: 100:0	AmD and AcoD				B-I: 0.27 NL g VS^{-1} B-II: 0.29 NL g VS^{-1} B-III: 0.29 NL g VS^{-1} B-IV: 0.23 NL g VS^{-1}	
Food waste (FW) and sewage sludge (SeS) (FW:SeS ratio, TS %)	Stage I: 100:0 Stage II: 100:0 Stage III: 75:25 Stage IV: 50:50 Stage V: 25:75 Stage VI: 0:100	AmD and AcoD	pH Biogas production Biogas composition Microbiology	Mesophilic (37 ± 2°C)	1.41 – 3.30 g VS $(L{\cdot}d)^{-1}$	I: 0.54 L g VS^{-1} II: 0.54 L g VS^{-1} III: 0.46 L g VS^{-1} IV: 0.37 L g VS^{-1} V: 0.31 L g VS^{-1} VI: 0.30 L g VS^{-1}	[43]

Feedstock	Feeding	Type of AD	Monitoring parameters	Temp.	OLR	Methane production	Ref.
Mixed sewage sludge (MSS) and fruit and vegetable waste (FVW) by a wholesale market (FVW:MSS ratio, , based on (v/v)) (S: summer; A: autumn; W: winter and Sp: spring)	Stage S-I: 0:100 Stage S-II: 20:80 Stage S-III: 40:60 Stage S-IV: 60:40 Stage S-V: 80:20 Stage S-VI: 100:0	AmD and AcoD	pH Alkalinity IA/PA ratio IA/TA ratio Biogas production Biogas composition % VS removal	Mesophilic (35°C)	0.6 to 5.5 g VS $(L{\cdot}d)^{-1}$	S-I: 0.276 L g VS^{-1} S-II: 0.346 L g VS^{-1} S-III: 0.33 L g VS^{-1} S-IV: 0.355 L g VS^{-1} S-V: 0.362 L g VS^{-1} S-VI: 0.323 L g VS^{-1}	[23]
	Stage A-I: 0:100 Stage A-II: 20:80 Stage A-III: 40:60 Stage A-IV: 60:40 Stage A-V: 80:20 Stage A-VI: 100:0				1.0 to 4.8 g VS $(L{\cdot}d)^{-1}$	A-I: 0.308 L g VS^{-1} A-II: 0.320 L g VS^{-1} A-III: 0.441 L g VS^{-1} A-IV: 0.447 L g VS^{-1} A-V: 0.409 L g VS^{-1} A-VI: 0.416 L g VS^{-1}	
	Stage W-I: 0:100 Stage W-II: 20:80 Stage W-III: 40:60 Stage W-IV: 60:40 Stage W-V: 80:20 Stage W-VI: 100:0				1.8 to 5.8 g VS $(L{\cdot}d)^{-1}$	W-I: 0.329 L g VS^{-1} W-II: 0.371 L g VS^{-1} W-III: 0.380 L g VS^{-1} W-IV: 0.418 L g VS^{-1} W-V: 0.405 L g VS^{-1} W-VI: 0.393 L g VS^{-1}	
	Stage Sp-I: 0:100 Stage Sp-II: 20:80 Stage Sp-III: 40:60 Stage Sp-IV: 60:40 Stage Sp-V: 80:20 Stage Sp-VI: 100:0				0.6 to 4.8 g VS $(L{\cdot}d)^{-1}$	Sp-I: 0.323 L g VS^{-1} Sp-II: 0.345 L g VS^{-1} Sp-III: 0.325 L g VS^{-1} Sp-IV: 0.340 L g VS^{-1} Sp-V: 0.348 L g VS^{-1} Sp-VI: 0.394 L g VS^{-1}	
Mixed sewage sludge (MSS) and fruit and vegetable waste (FVW) by a wholesale market (FVW:MSS ratio, based on (v/v))	Stage I: 0:100 Stage II: 20:80 Stage III: 40:60 Stage IV: 60:40 Stage V: 80:20 Stage VI: 100:0	AmD and AcoD	pH Alkalinity IA/PA ratio Biogas production Biogas composition % VS removal	Mesophilic (35 ± 1°C)	1.03 to 4.78 g VS $(L{\cdot}d)^{-1}$	I: 0.303 L g VS^{-1} II: 0.380 L g VS^{-1} III: 0.445 L g VS^{-1} IV: 0.405 L g VS^{-1} V: 0.390 L g VS^{-1} VI: 0.403 L g VS^{-1}	[26]

Feedstock	Feeding	Type of AD	Monitoring parameters	Temp.	OLR	Methane production	Ref.
Fruit and vegetable waste by a wholesale market (Fruit:Vegetable ratio fruit fraction %)	FVWs collected weekly with different % of fruit and vegetables. Cycle I: 36.89% fruit Cycle II: 52.83% fruit Cycle III: 44.62% fruit	AmD (Two steps/ pilot scale)	pH Alkalinity VFAs VFA/TA ratio Biogas production Biogas composition % VS removal	Mesophilic (35 ± 1°C)	0.5 to 4.06 g VS $(L{\cdot}d)^{-1}$	I: 0.35 NL g VS^{-1} II: 0.51 NL g VS^{-1} III: 0.55 NL g VS^{-1}	[4]
Source selected of organic fraction of municipal solid waste (SS-OFMSW) collected from a canteen and in a fruit and vegetable wholesale market, sewage sludge (SwS) from a WWTP thickener and treated wastewater (TW) (SwS:SS-OFMSW:TW ratios, weight-based)	Stage I: 100:0:0 Stage II: 90.9:1.5:7.6 Stage III: 90.9:3.0:6.1 Stage IV: 66.7:11.1:22.2 Stage V: 66.7:16.7:16.6 Stage VI: 41.3:29.3:29.4	AmD and AcoD	pH Alkalinity VFAs TAN Biogas production Biogas composition % VS removal % COD removal	Mesophilic (37–38°C)	I: 0.80 g VS $(L{\cdot}d)^{-1}$ II: 1.10 g VS $(L{\cdot}d)^{-1}$ III: 0.94 g VS $(L{\cdot}d)^{-1}$ IV: 1.23 kg VS $(m^3\ d)^{-1}$ V: 1.74 kg VS $(m^3\ d)^{-1}$ VI: 3.20 kg VS $(m^3\ d)^{-1}$	I: 0.25 NL g VS^{-1} II: 0.26 NL g VS^{-1} III: 0.32 NL g VS^{-1} IV: 0.32 NL g VS^{-1} V: 0.37 NL biogas g VS^{-1} VI: 0.49 NL biogas g VS^{-1}	[35]
Real digester effluente (RW from residual waste), waste paper plus cardboard (WP) and biowaste (BioW) diluted with digester supernatant from the plant (RW:BioW:WP ratios, based on (w:w))	Stage I: 100:0:0 Stage II: 0:100:0 Stage III: 0:85:15 Stage IV: 0:70:30	AmD and AcoD	Alkalinity VFAs VFA/TA ratio Biogas production Biogas composition % VS removal	Mesophilic (35°C)	2.9 ± 0.4 g VS $(L_R\ d)^{-1}$	I: 0.34 L g VS^{-1} II: 0.41 L g VS^{-1} III: 0.36 L g VS^{-1} IV: 0.34 L g VS^{-1}	[48]

Feedstock	Feeding	Type of AD	Monitoring parameters	Temp.	OLR	Methane production	Ref.
Fruit and vegetable waste (FVW) by a wholesale market (fruit:vegetable ratio, fruit fraction %)	Different fruit and vegetable mixtures with a fruit fraction of 33.4, 33.1, 28.4 and 60.6%	AmD (pilot scale)	pH Alkalinity VFAs VFA/TA ratio Biogas production Biogas composition % VS removal	Mesophilic (35 ± 0.5°C)	0.5 to 5.0 kg VS $(m^3\ d)^{-1}$	I: 0.47 NL g VS^{-1} II: 0.39 NL g VS^{-1} III: 0.44 NL g VS^{-1} IV: 0.46 NL g VS^{-1}	[34]
Fruit and vegetable waste from the central food distribution market and meat residues (MR) (FVW;MR ratios, based on (v/v))	Stage I: 100:0 Stage II: 75:25 Stage III: 50:50 Stage IV: 100:0 Stage V: 50:50 Stage VI: 75:25	AmD and AcoD	pH VFAs TAN Biogas production Biogas composition Microbiology % VS removal	Room temperature	2.4 to 2.7 g COD $(L\ d)^{-1}$	I: 0.10 L g VS^{-1} II: 0.14 L g VS^{-1} III: 0.12 L g VS^{-1} IV: 0.04 L g VS^{-1} V: 0.04 L g VS^{-1} VI: 0.14 L g VS^{-1}	[49]

AmD: anaerobic mono-digestion; AcoD: anaerobic co-digestion), where OLR, organic loading rate; VFA, volatile fatty acids; IA, intermediate alkalinity; PA, partial alkalinity; TA, total alkalinity; VS, volatile solids; TS, total solids; TAN, total ammonia nitrogen; and COD, chemical oxygen demand.

Table 2.
Composition of feedstock change.

On the other hand, in the research carried out by Fonoll et al. [48] and Scano et al. [34], the digestion systems were subjected to stressful scenarios to compare the robustness of the process with respect periods of stability. Fonoll et al. [48] have reported that 15% and 30% replacement of biowaste (BioW) with waste paper (WP) did not affect VFA and alkalinity levels. However, for a replacement of 30%, acidification of the supernatant used to dilute the feedstock led to a rapid accumulation of VFA (2400 mg L^{-1}), which decreased methane production. Recovery was carried out after a period without feeding and by re-establishing the feed supply using a new batch of supernatant. Scano et al. [34] observed an initial increase in VFA/TA, reaching values close to 0.65, corresponding to the increase in OLR due to a high percentage of fruit in the feed mixture. As a corrective strategy, the percentage of fruit in the mix was reduced, resulting in a corresponding reduction in VFA/TA. During the subsequent stages, VFA/TA was mainly influenced by chemical composition differences of the feed substrate, changes in OLR, and the simple sugar content of the fruit waste. Experimental results reported that the AD process still performed well with well-balanced mixtures of fruit and vegetable wastes, even for VFA/TA of up to 0.5. The highly elevated VFA/TA values (above 1) were derived from specific stress tests performed by feeding the reactor with substantial quantities of substrates with high content of simple sugars. The substrate mixture's large melon (which contains large amounts of highly degradable sugars) caused significant instability. Furthermore, it was complicated to stabilize the process at the next stage, as the available VWFs were mainly composed of fruit waste.

The assessment of the stability of the AD process when the feedstock composition changes through the TAN concentration measurement was reported by Cabbai et al. [35]. Organic nitrogen from the feed substrates (SS-OFSMW and SwS) was metabolized by the biomass-producing ammonia, although the levels were safe for process stability. García-Peña et al. [49], who evaluated the co-digestion of FVW by varying the percentage of meat residue (MR), reported that the addition of MT (75:25), rich in protein, started to release ammonia from the hydrolysis of the protein, which favored an increase in the alkalinity of the medium and the pH drop regulation.

Regarding methane production and composition, all the researchers have reported stable production values and related their differences to the characteristics and biodegradability of the feedstocks fed, just as to the different OLRs evaluated (**Table 2**).

In cases of feedstock composition change evaluating the microbial population adaptation, changes have been observed in contrast to feedstock-type changes. Tonanzi et al. [2] and Cheng et al. [43], who evaluated co-digestion of activated sewage sludge with percentage changes of FW, detected hydrolytic bacteria growth, such as *Bacteroidales*, especially the *Prolixibacteriaceae* family, whose relative abundance increased linearly with FW percentage in the mixture composition. As for the archaeal populations, high diversity indices were found when the FW and activated sewage sludge percentages in the feedstock composition varied, suggesting that the archaeal biodiversity was affected by the reactor feed conditions. Most of the acetoclastic methanogens determined belonged to the order *Methanosarcinales*, mainly to the genus *Methanosaeta*. In contrast, the hydrogenotrophic methanogens identified belonged to the orders *Methanomicrobiales* and *Methanobacteriales*, mainly to the genus *Methanobacterium*. The combined relative abundances of the three methanogens did not show significant changes in the two investigations. However, it was clear that *Methanosaeta* competed over *Methanobacterium*, and the latter had advantages with increasing FW in the feedstock composition. Furthermore, Tonanzi et al. [2] stated that minimal activated sewage sludge addition (FW: WAS, 95:5) enriched the microbial community with *Methanospirilloun* and *Candidatus Methanophastidiosun*,

which have a high H_2-consuming capacity, avoiding thermodynamic bottlenecks and failure of the FW mono-digestion process by the drop in activity of acetoclastic microorganisms as a result of a dramatic accumulation of propionic acid.

On the other hand, García-Peña et al. [49] reported enrichment of the microbial population of Firmicutes (fermenting bacteria that degrade VFAs), just to Bacteroidetes (proteolytic bacteria probably involved in the degradation of MR) when the percentage of MR increased in the composition of the raw material feeding. As for the archaea population, it was reported that the hydrogenotrophic methanogenic genus *Methanobacteriales* represented more than 93% of the presence of archaea in the digester. The hydrogenotrophic methanogenic community dominated even though the digester was inoculated with cow manure, which commonly contains acetoclastic methanogens. Finally, it may be argued that when changes are carried out to the composition of the feedstock fed with substrates of very diverse origins and biodegradability, microbial populations adapt to the changes in the end.

4. Conclusions

Different control strategies and parameters are reported in the literature to monitor system stability as a result of changes related to the feedstock. Several strategies have been implemented, reactor operating time extensions, lowering of OLRs, or supplementing enough alkalinity to buffer possible pH shocks. A longer operation time could allow the choice of a transition strategy to microbial community adaptation. Also, systems operation with low OLRs is less disturbed by instabilities. In any case, the transitory state is when the process needs to be carefully controlled not to reach a point of irreversible inhibition. The control parameters that should be monitored to prevent this situation would be pH and VFA/TA ratio. The microbial population analysis provides interesting information on its adaptation, although it does not reveal enough details for daily monitoring. Developing protocols for substrate changeover strategies could be of great interest to expand the use of existing anaerobic digestion facilities. It would improve the potential of this technology in the treatment of seasonal substrates that are generally not treated by anaerobic digestion. Concerning anaerobic digestion process robustness, changes in the type or composition of feedstock fed to the reactors are possible, although cautiously.

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Conflict of interest

The authors declare no conflict of interest.

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