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Mini- Review on Substrate and Inoculum Loadings for Anaerobic Co- Digestion of Food Waste

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Increasing production of food waste can lead to major environmental pollution if it is disposed without proper control in many countries. Food waste can be regarded as a resource rather than unwanted discard due to its high potential for resource recovery. Anaerobic digestion of food waste has shown promising potential for food waste treatment and valorisation by producing biogas as a renewable energy and digestate as fertiliser. Food waste has high biogas potential due to the presence of highly labile organic matter but this can lead to process instability. The process instability is often linked to the imbalance of process intermediates that affects the microbial community. Common parameters that are crucial for ensuring optimal metabolic activity of anaerobes includes temperature, pH, carbon-nitrogen ratio, organic loading rate, retention time and nutrient concentration. Co-digestion of food waste with other feedstocks are increasingly being practiced for better nutrient balance and reducing chances for rapid acidfication. The optimum conditions for the process has been shown to vary following different microbial inoculants and loadings of the respective substrates. This study aims to review only the effect of substrate and inoculum used during the AD of food waste, including the type of co-digested substrate, the mixing ratio, the microbial inoculant used and the substrate to inoculum ratio.

1. Introduction

Anaerobic digestion (AD) is a complicated and dynamical biological process that involved multiple physicochemical and biochemical reactions in sequential and parallel pathways (Pontoni et al., 2015). AD is characterised by four distinct phases, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. Some researchers have included a disintegration step before these four phases (Pontoni et al., 2015) where they categorised it into three phases, namely fermentation, acetogenesis and methanogenesis (Molino et al., 2013). The AD process is governed by different microbes with varied specific cell growth rates, substrate consumption capabilities and preferred environmental conditions, such as pH and temperature. As such suitable conditions, the AD allows the net production of thermal and electric energy, through avoided emissions and resource consumption (Lombardi et al., 2015).

In general, organic solid waste like food waste (FW) is managed by composting (Lim et al., 2016) or vermicomposting (Wu et al., 2014). AD of FW is a complex process that simultaneously digests all organic substrate in a single stage system (Zhang et al., 2014). Due to the high amount of labile organic matter (OM) present in the FW, the mono-digestion of FW often leads to process instability caused by rapid acid accumulation from the hydrolysis of labile OM. When the macromolecules such as carbohydrates, proteins and lipids are hydrolysed by anaerobes, volatile fatty acids (VFAs) are produced. VFAs, include acetate, propionate, butyrate and valerate, can be consumed by syntrophic acetogens and methanogens (Zhang et al., 2014). The proliferation of acid formers is usually faster and outrun the methane gas (CH_4) formers. If the acids production is greater than its consumption, the accumulated acids will lower the pH and lead to process failure as the optimal pH for methanogens is 6.5 - 7.8. FW is rich in protein and lipid if compared to other waste, like sewage sludge and agricultural waste. Degradation of protein produces ammonia-nitrogen (NH_3-N) which can either be

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as an essential nutrient for microbial growth or a toxic inhibiting methanogenesis (Chen et al., 2014). The microbes involved in AD are sensitive to the inhibitors, which can reduce the growth rates and disable the microbial activities (Reyes- Contreras and Vidal, 2015). Degradation of lipids produces long chain fatty acids (LCFAs) which is toxic to microbes. The inhibitory mechanism includes lipid flocs floating on the surface and LCFAs absorption onto the cell wall and membrane, thus interfering the mass transfer between the medium and microbes (Zhang et al., 2014). Albeit this inhibitory effect, high fat content in FW can give rise to high biogas yield. Theoretically, the hydrolysis of lipid will yield 1014 L CH_4/ kg VS where hydrolysis of protein and carbohydrates yield 496 L CH_4/ kg VS and 415 L CH_4/ kg VS respectively (Moller et al., 2004). Studies had reported the synergistic effect among NH_3, volatile fatty acids (VFAs) and LCFAs in maintaining the pH balance due to the buffering capacity. The inhibitory limits for these reactants thus varied in different studies. This paper reviewed the studies conducted on the AD of FW with special interest on the co-digested substrate, the effect of mixing ratio, the role of inoculum used and the substrate to inoculum ratio. This review provides a better insight for process design regarding the selection of substrate and inoculum to increase biogas yield. This reduces the need of process configuration such as pre-treatment, pH adjustment using alkaline, mechanical treatment, thermophilic reactor and multi-stage systems, which may exert significant requirement on cost and technical maturity in the developing countries.

2. Methods

A range of papers under Elsevier were reviewed based on the following keywords through the search engine of Science Direct: anaerobic digestion of food waste, anaerobic digestion of municipal solid waste, biogas production from food waste, biogas production from municipal solid waste, inoculum on anaerobic digestion, effect of substrate to inoculum on anaerobic digestion and effect of feed to microbes ratio on anaerobic digestion.

3. Co-digestion of Substrate and Mixing Ratio

To prevent inhibition, the OLR for mono- digestion of FW is either with low OLR or high hydraulic retention time (HRT), such as 2.25 kg VS/ m^3· d for the HRT of 80 d (Banks et al., 2011). FW or KW is high with VS, rich in carbohydrates, lipids and proteins that can be easily converted to VFAs, leading to accumulation of acids at earlier stage with significant pH drop if the system is lacking of sufficient buffering capacity (Veeken et al., 2000). The buffering capacity is complemented by the co- digested substrate. PM or cow manure (CM) is commonly used due to their high N content (NH_3 and NH_4^+) and high pH (~ 8) to complement FW with low pH and low bicarbonate alkalinity (Dennehy et al., 2016). At low pH, the main VFAs are acetate and butyrate that inhibit methanogenesis (Pantoni et al., 2015). The chances of VFAs inhibition increased following increased proportion of FW in the AD system. Inhibition was observed when FW proportion was at 20 % by Brown and Li (2013) or when KW proportion was greater than 26 % by Ye et al. (2013).

Substrate rich in N content is often used to adjust the nutrient content during AD of FW. C/ N is an important parameter and indicator of balanced nutrients. FW commonly has a C/ N ratio of 15- 20, some even lower. A C/ N ratio of 20 - 30 is deemed suitable for the AD process where the optimum value will vary upon type of waste used for co- substrate (Li et al., 2011) and the type of inoculum. Optimum C/ N ratio is important as study showed low utilisation of total C for methane production when total N is in excess (Wang et al., 2013).

Biogas composition is found to be varied with the chemical composition of feedstock used. A study by Liu et al (2009) showed that substrate mix with medium and high carbohydrates content but low and medium fat content respectively showed rapid degradation and no lag phase in biogas production. The system yielded a total biogas of 450 - 585 L/ kg VS and 508 - 617 L/ kg VS with a 50 - 60 % of CH_4. Another system with low and medium carbohydrates but high fat content produced 635 - 777 L/ kg VS of biogas, with 60.4 - 71.4 % of CH_4.

The papers reviewed are summarised in Table 1. The substrate used for co-digestion, the effect of mixing ratio of substrates, effect of substrate loading (organic loading rate, OLR) and respective biogas yield are presented. These substrates are known to be more non- readily biodegradable thus to balance against FW, which is rapidly biodegradable. Some exhibited higher pH which can be used to counter the acid accumulation produced by the rapid degradation of FW. The synergistic effect often led to low VFA inhibition and significantly higher production of biogas. Biogas is the mixture of CH_4 and CO_2, where the % CH_4 is used to indicate the biogas quality.

Table 1: Effect of feedstock mixing ratio during anaerobic co-digestion of FW on biogas yield and process stability.

Area	Substrate used	Mixing ratio of feedstock	Effect/ Remark	Biogas yield	Reference
USA	FW + YW	FW at 0 %, 10 %, 20 %	Reactor with more FW added experienced a delay in the peak production of biogas. Severe inhibition by VFAs at FW= 20 %.	8.6 L CH_4/ L at loading of 10 % FW with F/ I= 2.	Brown and Li, 2013
China	FW+ straw	5: 0, 1: 4, 1: 1, 3: 2, 4: 1, 5: 1, 6: 1, 7: 1, 8: 1	CH_4 yield increased by 39.5 % and 149.7 % compared to mono-digestion of FW and straw respectively.	392 L/ kg VS; 67.62 % CH_4.	Yong et al., 2015
China	RS+ KW + PM	0: 2: 1, 0.4: 1.6: 1, 0.8: 1.2: 1, 1.2: 0.8: 1, 1.6: 0.4: 1	Severe VFAs inhibition when KW addition is greater than 26 %; CH_4 production increased by 71.6 % compared to mono- digestion of RS.	674.4 biogas L/ kg VS added; 384 CH_4 L/ kg VS added	Ye et al., 2013
Ireland	FW+ PM	1: 0, 4: 1, 3: 2, 2: 3, 1: 4, 0: 1	Synergistic effect observed for all mixing ratio where no VFAs inhibition was observed.	521 L CH_4/ kg VS.	Dennehy et al., 2016
China	FW+ FVW + WAS	2: 1: 1 with progressive OLR	VFAs was observed to increase right after substrate addition but no obvious inhibition between OLR 1.2- 6.0 kg VS /m^3. d.	5.28 L/ L. d at OLR= 8 kg VS /m^3. d	Liu et al., 2012
Pakistan	FW+ RH	10.5: 1, 1.26: 1, 0.46: 1, 0.17: 1	Different mixing ratio to obtain four C/ N ratio= 20, 25, 30, 35; No obvious VFAs inhibition.	584 L / kg VS	Haider et al., 2015
Singapore	Brown water + FW	NA	-Higher CH_4 yield with co- digestion -No VFA inhibition	410 L/ kg VS	Rajagopal et al., 2013

Table 1 shows that co- digestion can effectively improve the AD performance with higher biogas yield. Mono-digestion is not preferable due to imbalance nutrients, slow starting up, long retention time and high possibility for process inhibition (Brown and Li, 2013).

4. Microbial Inoculant and Loadings during Co-digestion of Food Waste

Microbial inoculant (MI) has been used as a microbial booster to improve the AD of organic waste. Common MI includes the fresh dungs and anaerobically digested sludge from various wastewater treatment plants (WTPs) and manure treatment AD plants, where the microbes are more readily adapted to anaerobic condition. Wang et al. (2014) showed that inoculum obtained from anaerobic activated sludge performed better than aerobic activated sludge in terms of higher FW hydrolysis at any pH studied due to adaptation to anaerobic condition.

Certain amount of inoculum is needed for the substrate to provide sufficient initial microbial population. Higher amount of inoculum showed enhancement in the AD process by shortening the lag phase, enhancing VS removal and OM degradation (Elbeshbishy et al., 2012). The MI loadings with respect to substrate concentration is referred as food-to-microbial ratio (F/ M), substrate-to-inoculant ratio (S/ I) or food-to-inoculant ratio (F/ I). F/ I is used for this paper. The F/ I ratio is expressed either as the amount of feedstock added on VS basis per the amount of inoculum on the VS basis or per the amount of inoculum on the volatile suspended solid (VSS) basis (Liu et al., 2009).

The type of inoculum is important as they varied in microbial population and parameters such as total solids (TS), volatile solids (VS), pH and N content. Fresh dung generally contains more acidogens than methanogens if compared to the anaerobically treated sludge; over loading of fresh dung can cause imbalance between these two populations and lead to digestion instability. High loadings of fresh dung can lead to higher VFAs production that cannot be rapidly consumed by methanogens (Haider et al., 2015). Low number of methanogens due to low loadings of fresh dung can also lead to VFAs production due to low consumption from the limited methanogens (Zhou et al., 2011). It is difficult to access the exact concentration of NH_3 or VFAs to exert its inhibitory effect as the microbes can adapt and acclimate to the condition.

The use of acclimated microbes by feeding the MI with selected substrates has been effective to reduce the inhibition effect of high loadings of these substances (NH_3 or VFAs) on the microbial activities. Dennehy et al. (2016) fed the microbes with feedstock of 60 % FW and 40 % PM for 6 mth showed no inhibition in all mixing ratio even at a higher heavy metal concentration, with a F/I of 3: 1. Neves et al. (2009) used the effluent from a lab digester fed with CM and FW as MI found that at high substrate loadings of 12 g COD_{oil}/ $L_{reactor}$, after a lag phase of 10 d, the MI collected on 224th d started to mineralise the residual substrate, including the lipids or LCFAs observed or entrapped onto the biomass and fibres.

Table 2 presents a summary on the MI used for AD of FW from the literature, with their respective F/ I ratio and impact on the system performance.

Table 2: Used of MI and their loadings on the performance of co- AD for food waste.

Area	Substrate	MI used	F/ I ratio	Effect/ Remark	Reference
USA	FW+ YW	Effluent from mesophilic liquid of AD digested with FW, FOG, SS	F/ I= 1, 2, 3 against FW of 0 %, 10 %, 20 % at 36 °C	-Inhibition following increased F/ I ratio -Reactor experienced complete failure when F/ I ratio = 3; -Maximum volumetric production of 8.6 L CH_4/ kg $VS_{feedstock}$ at F/ I = 2, with 10 % FW addition.	Brown and Li, 2013
China	KW+ RS	Digested sludge	F/ I= 0.67	-Severe inhibition of VFAs was observed when KW in feedstock was larger than 26 %. -System performed well had propionate and acetate as dominant VFAs species while others had lactate, acetate and propionate.	Ye et al., 2013
Islamabad	FW+ RH	cow dung (fresh)	F/ I= 0.25, 0.5, 1.0, 1.5, 2.0	-FW and RS were mixed according to 10.5: 1, 1.26: 1, 0.46: 1 and 0.17: 1 to obtain C/ N of 20, 25, 30 and 35. -pH value remained high (> 7.5) along the process, indicating no serious inhibition of VFAs despite high proportion of FW in the system. - VFAs accumulation and lower biogas production at higher S/ I ratios, i.e. S/ I = 2, due to higher organic loadings and lower MI amount.	Haider et al., 2015
China	FW+ CM	Anaerobically treated activated sludge (acclimated for 14 mths)	N/ A	-Total CH_4 production increased by 41.4 % when FM: CM = 2; -Co-digestion was observed with a concentration of NH_3 exceeding the limit, i.e. > 700 mg/ L but no NH_3 inhibition occurred. -Attributed to high C/ N ratio and higher biodegradation of lipid due to dilution of CM.	Zhang et al., 2013
Ireland	FW+ PM	Acclimated microbes, 60 % FW and 40 % PM for 6 mths	F/ I= 0.33	- No VFAs inhibition for all mixing ratio - Co- digestion of substrate had higher Fe, Cu, Mn and Zn due to addition of PM; but no inhibitory effect was found except for conversion of butyric acid.	Dennehy et al., 2016
California	FW + GW	2 types: Sludge from mesophilic and thermophilic municipal WTP	Thermophilic condition: F/ I = 1.6, 3.1, 4.0, 5.0	- No significant difference in biogas production for F/ I= 1.6, 3.1 and 4.0. - At F/ I= 5.0, the system yielded lowest biogas and CH_4 content.	Liu et al., 2009

Referring to Table 2, for the increased amount of inoculum used, with lower F/ I ratio, it offers a positive effect on the system's performance and stability. Brown and Li (2013) compared the daily peak of CH_4 production at systems with 0 %, 10 % and 20 % FW addition. At 10 % FW addition, system with F/ I= 1 had 1.5 times increase in daily peak of CH_4 production despite of the delay in peak production as compared to system with F/ I= 2, which showed only 1.4 times increased in CH_4 peak production than system with 0 % FW. At 20 % FW addition, system with F/ I =1 showed 2.8 times increase in peak daily CH_4 production where system with F/I= 2 showed severe inhibition. At F/ I= 3, the system was found to fail completely.

Similar findings were found by Haider et al. (2015) demonstrated that a decrease in specific biogas yield following a lower amount of inoculum used. The study showed a 26 % decrease in inoculum amount, which is equivalent to an increase from F/ I= 0.25 to F/ I= 0.5, led to an 18 % decrease in the specific biogas yield. When the inoculum amount is further decrease to F/ I = 1.0, 52 % decrease in the specific biogas yield was observed. Liu et al. (2009) pointed out that biogas yield tends to decrease with higher F/ I ratios. They performed the AD at thermophilic (50 °C) with different F/ I ratio. For the mono- digestion of FW, the average biogas yield were 778, 742, 784 and 396 L/ kg VS at F /I= 1.6, 3.1, 4.0 and 5.0. Higher F/ I ratio with reduced inoculum added had lower methanogenic activity and less number of methanogens to consume the VFAs, thus leading to VFAs accumulation that further inhibited methanogenesis.

Proper selection of co-digestion substrate and use of acclimated microbes are effective strategies to overcome process inhibition and improve biogas yield. The VFAs/ alkalinity ratio is a crucial indicator of process stability. A ratio less than 0.4 is considered to be optimal for liquid AD and a ratio more than 0.6 indicated system overload. In the study by Brown and Li. (2013), the initial VFAs/alkalinity ratio was approximately 0.92 - 1.79, showing high VFAs accumulation but the initial pH were all greater than 6.8. For the final pH, most systems had a pH above 6.5 that is the minimum pH for AD to produce biogas (Li et al., 2014). The high final pH for most of the system despite of high VFAs accumulation could be due to the buffering capacity of yard waste and the use of acclimated microbes. Hidalgo et al. (2015) found that lower F/ I ratio affected the CH_4 production positively, where higher composition of pig manure led to shorter lag periods and lower HRT. Haider et al. (2015) further demonstrated that with high F/ I= 1.5 and 2.0, the drop in pH was slower than the system with low F/ I= 0.25. The smaller amount of inoculum used in the high F/I ratio and the presence of YW with high cellulosic content, had slower hydrolysis rate thus leading to slower acid production. The system is likely to experience VFAs inhibition as the number of methanogens is insufficient to consume VFAs for CH_4 production.

5. Conclusions

As a conclusion, the performance of AD for FW can be significantly improved by properly adjusting the mixing ratio of FW with substrate that can improve its buffering capacity against VFAs accumulation. CM or PM has been showed to be a good choice due to their high pH and high N content to buffer against the low pH from rapid acid accumulation and due to ease of FW hydrolysis. Substrates rich in non- labile OM, such as RS and RH, is a good selection as it can slow down the breakdown of labile OM and subsequent VFAs accumulation. Inhibition of VFAs has been observed when FW is more than 20 % in the system despite the addition of YW or RS. This might due to the choice of F/ I used. Systems with lower F/ I ratio showed no significant VFAs inhibition despite of the high loadings of FW but system with higher F/ I ratio, more than 0.5, showed a significant VFAs inhibition when FW addition is more than 20 %. Nevertheless, a lower amount of inoculum used could ensure a slower rate of hydrolysis, which gives a slower production of VFAs thus resulted in a delay in pH drop. This review reveals important strategies to increase the yield of methane gas for the AD of FW, which is rich in labile OM. Further studies is needed to better determine the inverse relationship of F/I ratio regarding VFAs production to determine the minimum F/ I ratio for a stable AD of FW. As such, more research is needed to identify a range of feedstock mixing ratio and their respective optimal F/ I ratio, to strategically improve the AD process of FW without the need of complex process configuration.

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