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REVIEW

Biohythane and organic acid production from food waste by two-stage anaerobic digestion: a review within biorefinery framework

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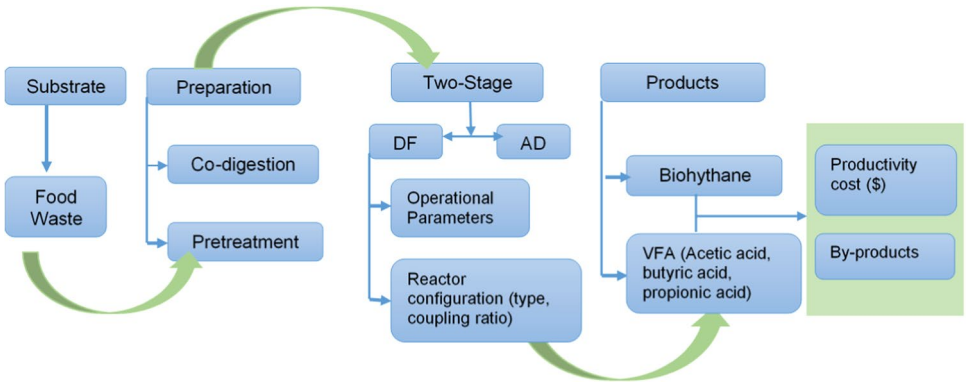
Abstract

The two-stage anaerobic digestion (AD) is gaining popularity because of the process stability and possibility of recovering multiple-resources such as biohydrogen and organic acids from the first stage dark fermentation (DF) and methane in the AD as the second stage while treating the organic waste. As the performance of two-stage processes is influenced by the type of substrate and operational conditions, there have been several experiments at laboratory and pilot scales to determine the optimum conditions. The main objective of this review is to provide an updated overview of advancements in biohythane and organic acids production from food waste (FW) in the two-stage DF-AD process. Likewise, this work also provides an insight into the economic and future prospective of utilizing organic acids for different biochemicals such as polyhydroxyalkanoates, polylactate, and microalgal biomass production. The integration of optimum operational parameters, pretreatment methods, types of bioreactors is essential in combined DF-AD processes. The parameters and reactor configuration have to be optimized depending upon the targeted end-products. More research into the techno-economic analysis of different bioreactor configurations for long-term operations in an integrated DF-AD process with FW as a feedstock is needed to realize its viability for commercial application.

Graphical abstract



Keywords Bioreactor configuration · Dark fermentation · Operational parameters · Production cost · Volatile fatty acid

Abbreviations

BESA	2-Bromoethasulfonic acid
AD	Anaerobic digestion
AF	Anaerobic fermentation
AMFR	Anaerobic membrane filtration reactor
CO_2	Carbon dioxide

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CSTR	Continuous stirred tank reactor
DF	Dark fermentation
EGSB	Expanded granular sludge bed
FW	Food waste
HRT	Hydraulic retention time
HCl	Hydrochloric acid
H_2	Hydrogen
HPP	Hydrogen partial pressure
LA	Lactic acid
LBR	Leachate bed reactor
LCA	Life cycle assessment
LCFA	Long-chain fatty acid
CH_4	Methane
NPV	Net present value
OFMSW	Organic fraction of municipal solid waste
OLR	Organic loading rate
PBR	Packed bed reactor
PHB	Polyhydroxy butyrate
PHA	Polyhydroxyalkanoates
PLA	Polylactic acid
KOH	Potassium hydroxide
RR	Recirculation ratio
SBR	Sequence batch reactor
SCFA	Short chain fatty acid
SRT	Solids retention time
NaOH	Sodium hydroxide
SCBL	Sugarcane bagasse and leaves
TS	Total solid
USD	United state dollars
UAPB	Up-flow anaerobic packed bed
UASB	Up-flow anaerobic sludge blanket
VFA	Volatile fatty acid
WAS	Waste-activated sludge

Introduction

With the increase in energy demand, depletion of fossil fuel, and growing concern over global warming, the interest in green technologies for resource recovery is increasing. The current global energy demand is expected to double by 2050. More than 80% of the current energy demand is fulfilled by fossil fuels which are limited resources. Moreover, the by-products from burning fossil fuels are the major source of greenhouse gas emissions. The need for renewable sources to produce bioenergy and biochemicals that have positive environmental impacts is driving the bio-based economy (Cherubini et al. 2009; Bastidas-Oyanedel et al. 2019). The bio-based economy promotes the technologies that recover energy and chemicals from biomass through the biological degradation process.

Anaerobic digestion (AD) is one of the widely used biological processes for the production of renewable energy as biogas and nutrients from organic waste (Jain 2019). The benefits of converting the combustible methane (CH_4) gas to heat and electricity through combined heat and power (CHP) plant and the use of digestate as fertilizer has made AD a popular technology. However, these factors may not upgrade both material recovery efficiency and the economic viability of commercial biogas facilities (Kaparaju et al. 2009; Sawatdeenarunat et al. 2015).

The pursuit of optimization of the AD process for multiple-resource recovery has led to realizing a two-stage AD process. The two-stage AD process differs from the single-stage AD in terms of microbial pathways, growth kinetics, and environmental condition for acidogens and methanogens which is carried out in two separate bioreactors. Besides, several studies show that the two-stage process is much more beneficial than single-stage processes in terms of performance, efficiency, and stability (Liu et al. 2006; Park et al. 2010; Luo et al. 2011; Ghimire et al. 2021). Moreover, the fermentation time and reactor volume are also reduced in a two-stage AD process with possibilities for multiple-resource recovery. As reported by Roy and Das (2016), the total gaseous energy recovery from the starchy wastewater for the two-stage process was 53.6% whereas only 28% of gaseous energy was recovered from the single-stage biohydrogen (H_2) production. The two-stage AD process also shows stability in the long run which opens up possibilities for upscaling (Cavinato et al. 2012; Massanet-Nicolau et al. 2015).

The two-stage AD process simultaneously produces H_2 in the first stage and CH_4 in the second stage. The combination of H_2 (10–25% v/v) and CH_4 (75–90% v/v) is termed biohythane which has been considered a high-grade fuel, better than methane in terms of flammability, flame speed, and easier ignition with less energy input (Bolzonella et al., 2019). In addition, the by-products from the two-stage AD process such as Volatile Fatty Acid (VFA), Lactic Acid (LA), and alcohol are useful biochemicals (O-Thong et al. 2018). The two-stage AD has a good potential to become a sustainable biorefinery approach for multiple fuels and biochemicals production. Various processes such as dark fermentation (DF), photo-fermentation (PF) within the two-stage biorefinery process are available for H_2 production. In particular, DF is the most studied and promising technology because it has the potential to produce H_2 along with biochemicals from a wide variety of feedstock (Nasr et al. 2012; Alibardi and Cossu 2016). Furthermore, combining DF with AD can be an appropriate option to create H_2 and CH_4, thus, biohythane (Meena et al. 2020).

Current research interests in "biorefinery" are growing as shown by an increasing number of publications of 2,153 scientific articles and book chapters in the last decade (2010–2020) compared to 402 numbers (before the decade) in Scopus. The research and commercial interest

in anaerobic biorefinery is increasing due to the versatility of the process for the production of multiple fuels and chemicals (K. C. et al. 2015; Agler et al. 2011; Tedesco and Stokes 2017). As such DF-based biochemical process can be a promising route for achieving the bio-based products and energy (Clomburg and Gonzalez, 2013; Bastidas-Oyanedel et al. 2015; Motte et al. 2015; Sarma et al. 2015; Luongo et al. 2017; Nizami et al. 2017; Moscoviz et al. 2018).

Conversion of Food waste (FW) to produce bioenergy (biofuels) and by-products (fertilizers, chemicals) has been considered as one of the efficient approaches for not only energy production but also solid waste management (Dahiya et al. 2018; Paritosh et al. 2017; Strazzera et al. 2018). About 57% and 53% of the waste composition is organic waste in low and middle-income countries, respectively (Kaza et al. 2018). The major portion of organic waste is FW. Globally, FW amounts to 1.6 billion tonnes and much of it ends up in landfill sites leading to the emission of greenhouse gases (GHGs). FW is estimated to produce 3.3 billion tonnes of carbon dioxide (CO_2) emissions per year (FAO 2013). The theoretical CH_4 yield and electricity production potential of FW is 0.300 m^3/kg VS and 473.8 kWh, respectively (Suhartini et al. 2019). Therefore, tapping the energy potential of FW via a biorefinery framework could be a sustainable approach for organic waste management and the production of multiple-resources for commercial use.

This paper aims to provide an extended overview of the DF and AD process for biohythane and biochemical production (particularly VFA) from FW within the biorefinery approach. This paper discusses how pretreatment, different operational parameters, and the choice of bioreactors in the DF-AD affect the substrate degradation and metabolic pathways for H_2, CH_4 and organic acids production from FW. Likewise, the optimum conditions for biohythane and VFA production, economic and future prospective of utilizing the VFA for different biochemical such as polyhydroxyalkanoates, polylactate, and microalgal biomass has been discussed.

FW as a feedstock for biohythane and biochemical production

The FW which is generally rich in carbohydrates has been widely used for H_2 and biochemical production. FW comprises mostly carbon chains and has a high energy content (Fatima et al. 2020)**.** The average energy content in the mixed food sample (meat, fruits, and vegetables) was found to be 14.31 MJ/kg (Tanai 2016). Every year, approximately one-third of the food produced for human consumption gets wasted which is likely to increase with the global population (Gustavsson et al. 2011). However, the characteristics of FW vary depending upon the consumption pattern of different countries (Paritosh et al. 2017). Globally, the highest FW constituent is cereal (more than 80,000 million tonnes) whereas considering only Asia, vegetable (60,000-kilo tonnes) contributes to the maximum FW (Paritosh et al. 2017). Thus, this abundant FW can be extensively used in two-step DF-AD (Bolzonella et al. 2019; Dahiya et al. 2018).

Generally, carbohydrate-rich FW is regarded as the most ideal substrate for H_2 and CH_4 production in a two-stage process, whereas lipids and protein-rich foods are the least preferred (Alibardi and Cossu 2016). A 20 fold increase in biological H_2 production was observed when using carbohydrate-rich substances compared to lipid and protein-rich substances (Fatima et al. 2020).

Cieciura-Włoch & Borowski (Cieciura-Włoch and Borowski 2019) found out that plant-based waste such as fruit and vegetable waste is most suitable for hydrogen production with the highest yield of 280 L H_2/kg VS. They also found out that slaughter house and kitchen waste have high methane yield but unfavorable for hydrogen production because of high protein and fat. The protein-rich substrate with a low C: N ratio increases ammonia concentration during the AD process leading to inhibition of methanogenic microorganisms (Bolzonella et al. 2019). Similarly, the lipid is degraded into long-chain fatty acids (LCFA) which have an inhibitory effect on acetogens and methanogens (Dasa et al. 2016).

Besides the nutrients, the high total solid (TS) content of around 20–30% in FW makes it favorable for operating the first stage reactor under dry conditions (Capson-Tojo et al. 2017). According to the TS content of the FW was also found to be adjusted to 4.3% and 10% in tests carried out by Algapani et al. (2018) and Akhlaghi et al. (2019), respectively. The TS content greater than 15% can result in a decrease in substrate conversion and lactate is produced as a major fermentation product (Ghimire et al. 2017). Similarly, methane yield was found to be higher in the FW of TS 15–20% than in the TS of 5–10% (Chen et al. 2014). FW has a high VS content (21 to 27% VS) and is particularly suitable for the co-production of biohydrogen and platform molecules such as short organic acids and/or alcohols (Uçkun Kıran et al. 2015).

FW can be co-digested with substrates such as anaerobic sludge (can function as inoculum), waste activated sludge, wheat straw, chicken manure, and grass to enhance biohythane and VFA production (Ghimire et al. 2017; Show et al. 2018; Wang et al. 2014; Esteban-Gutiérrez et al. 2018). According to Wang et al. (2014), the VFA production from FW with the addition of aerobic and anaerobic activated sludge was recorded to be 0.482 g/g $VSS_{removal}$ and 0.918 g/g $VSS_{removal}$, respectively. Similarly, the co-digestion of FW with brown water (feces without urine) formed acetic and butyric acids as major metabolites which are the most

appropriate for methe and useable by-products production (Rajagopal et al. 2014).

FW is rich in organic content due to which there is a possibility of hydrolysis inhibition by the accumulation of ammonia, rapid acidification, and formation of LCFA (Menzel et al. 2020). This inhibits the optimum energy recovery from FW in single-stage AD. Therefore, besides co-digestion, two-stage AD, and pretreatment have also been considered as the efficient approach for enhancing the solubilization and hydrolysis of FW (Uçkun Kiran et al. 2015; Rodríguez-Valderrama et al. 2020; Menzel et al. 2020).

Two-stage AD process as a pathway for biohythane and biochemicals

Generally, the conversion of organic waste during AD consists of four steps: (i) hydrolysis (ii) acidogenesis (iii) acetogenesis, and iv) methanogenesis. In the two-stage AD process, these processes are physically separated by two reactors under controlled operating conditions (Fig. 1).

Stage I: dark fermentation (DF) process

Hydrolysis and acidogenesis phases are carried out in the first stage reactor. In this reactor, the complex organic compounds are hydrolyzed and further degraded by acidogenic microorganisms to produce C2-C5-based VFA, H_2, CO_2, and alcohols in the DF process (Camacho et al. 2019). Even though FW is readily biodegradable, hydrolysis is the rate-limiting step during the AD process (Zhang et al. 2014). However, for the two-stage AD process of organic waste, methanogenesis is the rate-limiting step (Meena et al. 2020) because of the slower methanogenic kinetics and longer lag phase in the second-stage reactor (De Gioannis et al. 2014).

During the DF process, most of the energy contained in the organic substrate is converted into VFAs whereas only 7.5–15% of the total energy is converted to H_2 (Bolzonella et al. 2019). The possible pathways for H_2 production are presented in Table 1 Eqs. (1) to (4), which shows

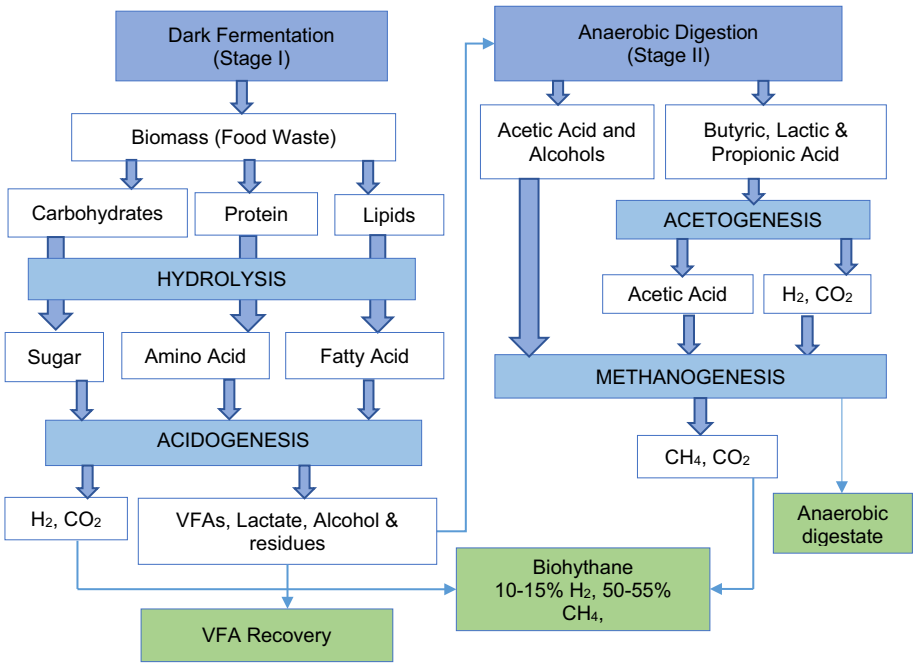

Fig. 1 Schematic diagram of the two-stage AD process

Table 1 Equation representing metabolic pathway for H_2 production

Possible H_2 producing pathways	Metabolic pathway	$\Delta G^{'}_{0}$ [a] (kJ/mol)	Equation
$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$	Acetate	− 206.3	(1)
$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$	Butyrate	− 254.8	(2)
$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2OH + CH_3COOH + 2CO_2 + 2H_2$	Ethanol & acetate	− 215.7	(3)
$4C_6H_{12}O_6 + 2H_2O \rightarrow 3CH_3CH_2CH_2COOH + 2CH_3COOH + 8CO_2 + 10H_2$	Butyrate & acetate	− 254.0	(4)

[a] $\Delta G^{'}_{0}$ values are adapted from (Thauer et al. 1977; S.-H. Kim et al. 2006a, b)

that the maximum theoretical yield from the DF process can be 4 mol H_2/mol glucose (Saratale et al. 2019). However, this cannot be achieved due to the complex nature of the FW which may contain a different fraction of carbohydrates, proteins, and fat.

Stage II-anaerobic digestion (AD) process

The DF effluent from the first stage can be fed into the second stage in which acetogenesis and methanogenesis occur. The VFAs with higher molecular weight and other intermediates are further oxidized by H_2-producing acetogens as shown in Eqs. 5–7 (Van et al. 2020).

$$CH_3CH_2COO^- + 2H_2O \leftrightarrow CH_3COO^- + CO_2 + 3H_2 \quad (5)$$

$$CH_3(CH_2)_2COO^- + 2H_2O \leftrightarrow 2CH_3COO^- + 2H_2 + H^+ \quad (6)$$

$$CH_3CH_2OH + H_2O \leftrightarrow CH_3COO^- + 2H_2 + H^+ \quad (7)$$

Approximately, 25% of acetate and 11% of H_2 are formed in acetogenesis (Anukam et al. 2019). Though H_2 is produced during acetogenesis, it is not recommended to extract H_2 from this stage (Van et al. 2020). Because acetogenic microorganisms are compatible with methanogens (Roy and Das 2016). And, this acid phase product (acetate and H_2 and CO_2) is consumed by methanogenic microorganisms for CH_4 production (Meegoda et al. 2018). The undigested or unhydrolyzed food residues can also be converted to CH_4 in the second-stage reactor. In the two-stage AD process, AD is mostly used for the recovery of CH_4 rather than organic acids.

In acetoclastic methanogenesis, acetate is converted into CH_4 and CO_2 which is accountable for two-thrid of CH_4 production (Eq. 8). Alternatively, reduction or CO_2 to CH_4 can also take place, which is termed as hydrogenotrophic methanogenesis which is accountable for the remaining one-third CH_4 (Eq. 9) (Fenchel et al. 2012).

$$CH_3COOH \rightarrow 2CO_2 + CH_4 \quad (8)$$

$$4H_2 + 2CO_2 \rightarrow CH_4 + H_2O \quad (9)$$

The physical separation benefits the growth of required microorganisms within that particular process. For example, hydrolysis is limited by fermentative bacteria whose optimum pH is 5.5, whereas the pH of 7.5–8.5 is needed for methanogenic microorganisms (Sivagurunathan et al. 2018). In DF, the hydrolysis and acidogenesis process is maintained by lowering the hydraulic loading rate (HRT), controlling the pH around 5.5—6.5, and inoculum pretreatment to enrich H_2 producers (Chu et al. 2008; Xia et al. 2016; Rafieenia et al. 2017).

Operational parameters affecting the two-stage AD process producing biohythane.

The optimization of operational parameters such as pH, temperature, hydraulic retention time (HRT), substrate loading rates, pretreatment methods, recirculation of effluent from the second stage to the first stage (recirculation ratio), types of bioreactor is essential in the integrated two-stage DF-AD process. The operational parameters affecting the biohythane production in the two-stage AD process are shown in Fig. 2. The effect of varying operating parameters in a two-stage AD process has been summarized and presented in Table 2.

pH

The pH is one of the most important parameters in biohythane production because it determines the subsistence of acidogenic or methanogenic microorganisms. It directly affects the metabolic pathways involved in the process of anaerobic fermentation and digestion. The formation of undissociated organic acids when pH is lower than 4 ceases H_2 production in DF (Bolzonella et al. 2019).

While operating a two-stage process for biohythane production from FW, Cavinato et al. (2011) reported 15% of H_2 production in the first phase and 65% of CH_4 in the second phase at pH of 4.32 and 7.68, respectively. Moreover, around 35% of H_2 production was observed at pH 3.51. Maintaining pH at acidic and neutral alkaline conditions is suitable for DF and AD reactors, respectively. This creates a favorable growth environment for H_2 producing microorganisms in DF and methanogens in AD (Meena et al. 2020).

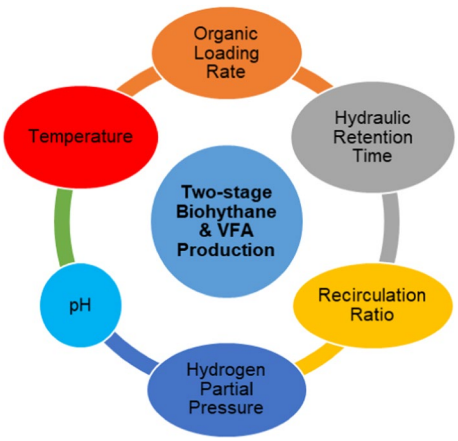

Fig. 2 Operating parameters affecting the biohythane production

Table 2 Two-stage AD process using FW under varying operating conditions

Substrate	Co-substrate	Pretreatment of FW/ inoculum	1st Stage	2nd Stage	Hydrogen Yield/ Productivity (L/ kg VS)	Methane Yield/ Productivity (L/ kg VS)	Energy Production (MJ/kg VS)	References	Limitation
FW	AD sludge		Type: CSTR Capacity:10 L pH: 5.5	Type: CSTR Capacity: 40 L	205	464	-	Chu et al. (2008)	The study indicated that the pH and temperature controlled two-stage AD process is economical and enviornment friendly. Calculation on the economical and environmental friendly parameters would have helped to confirm the benefits
			Temp. (°C): 55 OLR: 38.4 gVS/L/d	pH: 7.5					
			HRT: 1.3 days RR: 2	Temp. (°C): 35					
				OLR: 16.3 g VS/L/d HRT: 5 days					
FW	-	Heat treatment of FW at 100 °C	Type: Fermentation tank (pilot) Capacity:500 L pH: 5.3 ± 0.2 Temp. (°C): 33 ± 1 HRT: 2.75 days OLR: 7.4 g COD/L/d	Type: UASB Capacity:2300 L pH: 7.4 ± 0.3	0.62 L/m^3/d	-		Lee and Chung (2010)	The study considered the overall two-stage AD process from the storage unit, the energy production and purification unit to evaluate its economic viability. But, the effect of different composition of supernatant liquid, storage time for the FW, microbial activities for H_2/CH_4 production is not well addressed in the study

Table 2 (continued)

Substrate	Co-substrate	Pretreatment of FW/ inoculum	1st Stage	2nd Stage	Hydrogen Yield/ Productivity (L/ kg VS)	Methane Yield/ Productivity (L/ kg VS)	Energy Production (MJ/kg VS)	References	Limitation
				Temp. (°C): 36 ± 4 HRT: 6.4 days OLR: 1.65–3.9 g COD/L/d					
FW	AD sludge	-	Type: CSTR Capacity: 2 to 5 L pH: 5.5	Type: CSTR Capacity: 10 L	117 ± 14	311 ± 35		Chinellato et al. (2013)	The experiment was conducted under constant HRT, changing OLR and RR. The two-stage AD was not deemed feasible at high OLR. Additional experiment with the combination of low HRT and high OLR could have been conducted to determine the change in pH and the required RR for that condition
			Temp. (°C): 55 OLR: 20 gVS/L/d HRT: 1.3 days RR: 2.9	pH: 5.5					
				Temp. (°C): 55 OLR: 20gVS/L/d HRT: 1.3 days					

Table 2 (continued)

Substrate	Co-substrate	Pretreatment of FW/ inoculum	1st Stage	2nd Stage	Hydrogen Yield/ Productivity (L/ kg VS)	Methane Yield/ Productivity (L/ kg VS)	Energy Production (MJ/kg VS)	References	Limitation
Segregated OFMSW*	Granular Sludge from brewery; WAS	Heat	Type: Batch Capacity: 1 L	Type: Batch Capacity: 1 L	85 ± 3 mL/gVS/d	430 ± 11 mL CH_4/ gVS/d		Alibardi and Cossu (2015)	The study primarily focused on the effect of organic contents (carbohydrate and lipid) of FW on H_2 and CH_4 production. The effect of protein-rich FW and the process parameters on H_2-CH_4 production and stability of the system is not studied
			pH: 5.5	pH: 7.5					
			Temp. (°C): 35 ± 1 Heat treatment on inoculum	Temp. (°C): 35 ± 1 No heat treatment					
Synthetic FW	Digested sludge from AD	-	Type: Semi-continuous Capacity: 1.6 L pH: 5	Type: AFBR Capacity:1.3 L pH: 7.2–7.7	115.2 ± 5.3	334.7 ± 18.6	12.3	Yeshanew et al. (2016)	Integration of CSTR and AFBR has performed well even at the lowest HRT (1.5 days) at the laboratory scale. However, the HRT in the first stagecan be further decreased below 6 days as reported in many studies
			Temp (°C): 55 HRT: 6 days OLR: 1 kg VS/ m^3/d	Temp: 37					
				HRT: 20 days					

Table 2 (continued)

Substrate	Co-substrate	Pretreatment of FW/ inoculum	1st Stage	2nd Stage	Hydrogen Yield/ Productivity (L/ kg VS)	Methane Yield/ Productivity (L/ kg VS)	Energy Production (MJ/kg VS)	References	Limitation
FW	-		Type: CSTR Capacity: 200 mL pH: 5–6 Temp (°C): 55	Type: CSTR Capacity: 760 mL Temp (°C): 55 OLR: 4.8 kg TVS/ m^3/d	66.7	428 L/kgVS/d	-	Cavinato et al. (2012)	The study recommends multivariate model to predict ammonia relating to alkali and conductivity at real-time. Further study is necessary to understand the stability and certainty of multivariate model in two-stage AD process at long run
			OLR: 16.3 kg TVS/m^3/d	HRT: 12.6 days					
			HRT: 3.3 days						
FW	Activated sludge + Methanogenic sludge	-	Type: Batch Capacity: 2L	Type: Batch Capacity: 5 L	56.5	392	14.5	De Gioannis et al. (2017)	The study compared 2-stage and 1-stage AD in terms of energy recovery. The energy input and net energy gain for 2-stage and 1-stage AD needs to evaluated to compare the benefits
			SI: 1/0.14	SI: 1/2					
			pH: 6.5	Temp. (°C): 39 ± 1					
			Temp. (°C): 39 ± 1						

Table 2 (continued)

Substrate	Co-substrate	Pretreatment of FW/ inoculum	1st Stage	2nd Stage	Hydrogen Yield/ Productivity (L/ kg VS)	Methane Yield/ Productivity (L/ kg VS)	Energy Production (MJ/kg VS)	References	Limitation
FW	Seed inoculum	Hydrothermal pretreatment (140 °C)	Type: Batch Capacity: 417 mL pH: 6 ± 0.1 Temp (°C): 35 Run-time: 48 h	Type: Batch Capacity: 417 mL pH: 6 ± 0.1 Temp (°C): 35 SI: 1:2 Run-time: 24 days	43.1	511.6	18.8	Ding et al. (2017)	Hydrothermal pretreatment of FW contributed to better energy production. However, the energy input required for hydrothermal pretreatment and thus, the net energy yield could have also been calculated to determine its suitability
FW	Granular sludge	DF: Aerobic pretreatment AD: Untreated inoculum	Type: Batch Capacity: 1L Temp. (°C): 35 Pre-treated inoculum	Type: Batch Capacity: 1 L Temp. (°C): 35 Untreated inoculum	5.3	351	12.6	Rafieenia et al. (2017)	The air flow rate for aeration pretreatment of FW was fixed at 5 L/h for 24 h. The study on different air flow rates and time duration could have given more idea on the efficiency of aeration pretreatment of FW
FW	Effluent from thermophilic AD plant treating maize straw and mesophilic AD plant treating sewage sludge	-	Type: CSTR Capacity: 4.5 L Temp. (°C): 55 pH:5–5.5 HRT: 5 days OLR: 16.3 kgVS/ m3.d	Type: CSTR Capacity: 4.5 L Temp. (°C): 35 pH:5–5.5 HRT: 15 days	104.5	526 ± 137.2	22.7	Algapani et al. (2018)	The significance of adding trace metals in CH_4 reactor is not described well

Table 2 (continued)

Substrate	Co-substrate	Pretreatment of FW/ inoculum	1st Stage	2nd Stage	Hydrogen Yield/ Productivity (L/ kg VS)	Methane Yield/ Productivity (L/ kg VS)	Energy Production (MJ/kg VS)	References	Limitation
FW	Effluent from thermophilic AD plant treating maize straw and mesophilic AD plant treating sewage sludge	-	Type: CSTR Capacity: 2L	Type: CSTR Capacity: 4.5 L	135	510	20.5	Algapani et al. (2019)	The RR reduced the amount of alkali added in the first reactor to maintain pH. The influence of higher recirculation rate without the alkali addition on H_2 and CH_4 production could have also been explored
			pH: 5.5	pH: 7.8					
			Temp. (°C): 55 HRT: 5 days	Temp. (°C): 35					
			OLR: 16.3 kg VS/m^3/d	HRT: 15 days					
			RR: 0.3	OLR: 5.7 kg VS/ m^3/d					
FW	Effluent from thermophilic AD plant treating maize straw and mesophilic AD plant treating sewage sludge	-	Type: CSTR Capacity: 2 L	Type: CSTR Capacity: 4.5 L	125 ± 59	-	22.6	Algapani et al. (2019)	
			pH: 5.5	pH: 7.8					
			Temp. (°C): 55 HRT: 5 days OLR: 16.3 kg VS/ m^3/d	Temp. (°C): 35 HRT: 15 days					
				OLR: 5.7 kg VS/ m^3/d					

Table 2 (continued)

Substrate	Co-substrate	Pretreatment of FW/ inoculum	1st Stage	2nd Stage	Hydrogen Yield/ Productivity (L/ kg VS)	Methane Yield/ Productivity (L/ kg VS)	Energy Production (MJ/kg VS)	References	Limitation
FW	Anaerobic digestate	DF: Heat-treated inoculum at 105°C; AD: Untreated inoculum	Type: Batch Capacity: 1 L pH: 5.5 Temp. (°C): 55	Type: Batch Capacity: 1 L pH: 7 Temp. (°C): 55	37.6 ± 1	307.5 ± 10	11.4	Ghimire et al. (2021)	The study compared the energy yield from DF-AD process at thermophilic and mesophilic condition. Assessing the energy input for the system and net energy gain could indicate the viability for commercial application
FW	Anaerobic digestate	DF: Heat-treated inoculum at 105°C; AD: Untreated inoculum	Type: Batch Capacity: 1 L pH: 5.5 Temp. (°C): 35	Type: Batch Capacity: 1 L pH: 5.5 Temp. (°C): 35	53.5 ± 4	276.5 ± 4.3	10.4	(Ghimire et al. 2021)	

* OFMSW: Organic Fraction of Municipal Solid Waste; SI: Substrate to Inoculum ratio

Anaerobic fermentation (AF) or DF process have been used by the different researchers for VFA production under controlled pH varying from pH of 6 to 8 (Table 3). However, the VFA production was improved in alkaline conditions (pH 9) in comparison to acidic conditions (pH 6) for FW in batch tests (Cheah et al. 2019). The certain value of pH also affects the concentration of targeted VFAs. The culture pH of 6 was found to be suitable for the production of acetate whereas, pH 7 facilitated the production of butyrate from FW (Hussain et al. 2017).

The pH inside the first stage keeps on fluctuating due to the formation of VFAs. This inhibits the H_2 production pathway as H_2 and CO_2 is consumed by acetogens to produce acetate (Cavinato et al. 2011). Therefore, the pH of the first stage reactor can be controlled by external chemical addition (Micolucci et al. 2014), the use of co-substrate with high alkalinity or buffering capacity (Yeshanew et al. 2016; Ghimire et al. 2016), and continuous operation for CH_4 production could also avoid pH drop as high VFA concentration is buffered by high total ammonia concentration (Capson-Tojo et al. 2016). However, these external methods and continuous processes result in high costs and instability of operation. Therefore, a cost-effective solution in two-stage AD could be recirculating the digestate of the CH_4 reactor to the H_2 reactor (Cavinato et al. 2012, 2011; Micolucci et al. 2014).

Temperature

An optimum temperature plays a vital role in the growth of microorganisms as it influences biochemical reactions and their metabolism. Microorganisms that are responsible for H_2 production in the first stage and the CH_4 production in the second stage are often found to be grown at mesophilic (30–35 °C) to, thermophilic (55–60 °C) to higher thermophilic condition (70–90 °C) (O-Thong et al. 2018).

Most of the researchers have kept the optimum temperature at 55 °C for biohythane production as evident in Table 2. According to O-Thong et al. (2018), higher thermophilic temperatures are more favorable for H_2 production during DF than thermophilic and mesophilic conditions. Microorganisms such as *Caldicellulosiruptor* sp. survive in extreme temperatures and have hydrolytic enzymes which can utilize various substrates such as cellulose for more H_2 production. Moreover, higher temperature accelerates their metabolism and degradation efficiency that reduces the retention time for biohythane production. Furthermore, thermophilic condition decreases the solubility of CH_4 and CO_2 along with the destruction of pathogens (O-Thong et al. 2018). However, Ghimire et al. (2021) recorded the highest H_2 (53.5 mL H_2/g VS_{added}) and CH_4 (307.5 L CH_4/kg VS_{added}) production from FW in the DF-AD process at mesophilic and thermophilic temperature, respectively. The probable reason for this distinction is the quicker adaptation of heat-treated inoculum at the mesophilic condition as it is sourced from the mesophilic digester (Ghimire et al. 2021).

The most commonly used process temperature for VFA production from the AF process is 35℃ (Table 3). According to Cho et al. (2015), the VFA concentration from AF increases with the increase in temperature. VFA produced at 55 °C was 3.2 times greater than the VFA produced at 35 °C. Similarly, the concentration of propionic acid increased by 15% but acetic acid decreased to 65.8% with the increase in temperature. However, Jiang et al. (2013) found 45℃ as the most favorable temperature for the highest VFA production (47.89 g/L). The value was only slightly higher than the VFA (41.34 g/l) produced at 35 ℃. According to Jiang et al. (2013), the higher temperature leads to higher solubilization but lower acidogenesis of FW thus, leading to lower VFA production.

Hydrogen partial pressure (HPP)

The hydrogen partial pressure (HPP) in the liquid phase is an essential parameter for the DF (Ding and Zhao 2018; Beckers et al. 2012). Because high HPP (> 60 Pa) shifts the equilibrium reaction to the H_2 producing reaction (Ding and Zhao 2018) by deactivating the enzymes such as hydrogenase and NADH (Ramírez-Morales et al. 2015). However, no correlation was reported between HPP, liquid, and the $NADH/NAD^+$ ratio (De Kok et al. 2013). But, lowering HPP affected pH and microbial growth rate in DF (Ding and Zhao 2018). The H_2 and VFA production in DF decreases with the increase in HPP (Ramírez-Morales et al. 2013). Besides the concentration, HPP also influences the composition of VFAs though propionic acid concentration is not directly affected by ppH_2 (Ding and Zhao 2018).

A study conducted by Beckers et al. (2012) presented an increase of 7% of biohydrogen yields on the decrease total pressure 0.11 bar and a decrease of 19.5% yield on the lowering of pressure by 0.18 bar. The researchers experimented on an anaerobic biodisc reactor (ABR) that fixes the biomass and favors liquid to gas transfer leading to efficient H_2 production (Beckers et al. 2012). An identical study by Cazier et al. (2015) altered HPP from 0 to 1.557 bar which led to a substantial decrease in CH_4 yield and substrate degradation. It was seen that high HPP led to a decrease in degradation of the substrate as well as accumulation of metabolites.

The HPP can be controlled by venting out excess H_2 gas. Another solution could be introducing different gases such as N_2, CO_2, CH_4, or even biogas and creating a vacuum on the headspace of the reactor (De Kok et al. 2013; Dionisi et al. 2019). However, this leads to diluted H_2 production. Ramírez-Morales et al. (Ramírez-Morales et al. 2015) suggested integrating membrane technology to remove excess

Table 3 Production of VFA from different types of fermentation

Feedstock	Inoculum	Reactor types, volume	Operating conditions	Targeted organic acids	Total Organic Acid/ VFA Production	Reference	Limitation
FW		Acidogenic Fermentation tank, 500 L	pH: 5.3 ± 0.2 Temp. (°C): 30 ± 2 HRT: 2.75 days OLR: 7.4 g COD/L.d	VFA; AA; PA; BA	6576 ± 920 (3143 ± 583[a]; 903 ± 284[b]; 1444 ± 229[c]) mg/L	Lee and Chung (2010)	The study showed concentration of different organic acids under the selected operating conditions of HRT and OLR. The influence of the operating parameters on the metabolic pathways for the production of different organic acids needs to be further elucidated
Kitchen waste	Waste activated sludge	-	Controlled pH 8, 37 °C, C:N 22, 6 days HRT	Short Chain Fatty Acid (SCFA)	692.4 mg/g VS	Chen et al. (2013)	In this study, the optimum condition for the SCFA production from co-fermentation of FW with WAS using NaOH for alkaline condition was presented. Further comparison using Ca $(OH)_2$ to create alkaline condition could be done in terms of SCFA production and cost
FW	Anaerobic digested sludge	Batch, 4500 ml	Controlled pH 6, 35 °C	VFA	316 mg/g VS	Jiang et al. (2013)	The study showed the relationship of increase or decrease in VFA production from FW in accordance to pH, temperature and OLR. However, how these parameters influence the microbial process and stability of the system needs to be further investigated

Table 3 (continued)

Feedstock	Inoculum	Reactor types, volume	Operating conditions	Targeted organic acids	Total Organic Acid/ VFA Production	Reference	Limitation
FW	Anaerobic digested sludge	CSTR (Semi-continous), 4500 ml	Controlled pH 6, Constant temperature 45 °C	VFA	690 mg/ g VS	Jiang et al. (2013)	
FW	Anaerobic digested sludge	CSTR (Semi-continous), 4500 ml	Controlled pH 6, Constant temperature 35 °C, HRT 5 days, OLR 5 g TS/L d	VFA	504 mg/ g VS	Jiang et al. (2013)	
FW	Anaerobic activated sludge	Batch, 500 ml	Controlled pH 6.0, 30 °C, TS: 7%, S:I-4:1	VFA	918 mg /g VS removed	Wang et al. (2014)	The effect of pH on VFA production from FW using inoculum from different sources was studied. The influence of microbial community present in the inoculum on VFA production is not explained well
FW	Waste activated sludge	Batch, 500 ml	Controlled pH 6.0, 30 °C, TS: 7%, S:I-4:1	VFA	482 mg/ g VSS removed	Wang et al. (2014)	
FW	brown water	Automoatic Methane Potential Test System (AMPTS), 500 mL	Uncontrolled pH (initial 6.2–2.5), 35 ± 1 °C, fermaentation time 6 days	VFA	12.2 g/l	Rajagopal et al. (2014)	The co-digestion of FW and brown water under a) no inoculum, no pH control b) no inoculum but pH control c) inoculum addition but no pH control. But, the significance and pathway for the conversion of non VFA-COD to VFA-COD needs to be studied

Table 3 (continued)

Feedstock	Inoculum	Reactor types, volume	Operating conditions	Targeted organic acids	Total Organic Acid/ VFA Production	Reference	Limitation
FW	Anaerobic activate sludge	500 mL	Controlled pH 6, 30 ± 2 °C, 7% TS, Fermentation time 15 days	VFA	908 mg/gVSS removed	Yin et al. (2014)	VFA production is inhibited at high hydrothermal pretreatment of FW which is attributed to the inhibitory by-products. The change in metabolic pathways and chemicals created after the hydrothermal pretreatment needs to carriedout
FW	Anaerobic digested sludge	-	Controlled pH 6.0, 35 °C	VFA	799 mg /g VS	Wang et al. (2015)	The effect of initial TS content of combined FW and inoculum on VFA production was studied. Varying TS% only in FW would have given a better understanding on how it affects the acidification process
Simulated food and vegetable waste	Seed sludge from anaerobic digestor	CSTR, 2000 ml	Controlled pH 4 ± 1, 35 °C, SRT: 3 days, OLR: 11gVS/L/d	Lactic acid	10–20 g/l	Wu et al. (2015)	Long-term stability of lactic acid formation from acidogenic fermentation of FW at a single OLR and SRT is demonstrated. The influence of varying OLR and SRT could give a better understanding of stability of lactic acid production

Table 3 (continued)

Feedstock	Inoculum	Reactor types, volume	Operating conditions	Targeted organic acids	Total Organic Acid/ VFA Production	Reference	Limitation
FW	Homogenized thermophilic anerobic sludge	Leach Bed Reactor, 10 L	pH 7, 50°, IS: 4% (dry basis), leachate recirculation rate 2.6 L/L/d	VFA	247 mg/g TVS	Hussain et al. (2017)	The study showed increased VFA production with increased recirculation rate. The relation between soluble substrate and distribution of acidogenic microorganism with leachate recirculation rate needs to be further explored
FW and sewage sludge	Anaerobic digestate	Batch, 2 L	Controlled pH 7.20–7.60, Temp. (°C): 37 °C, 5% TS	VFA	281.84 mg/g VS	Li et al. (2018)	The underlying mechanism for VFA production co-digesting FW with sewage sludge is explored. The effect of adding inoculum to substrate ratio at 10% on VFA production also needs to be studied
FW	Effluent from thermophilic AD plant treating maize straw and mesophilic AD plant treating sewage sludge	CSTR, 2 L	pH: 5.5 Temp (°C): 55 HRT: 5 days OLR: 16.3 kg VS/m3.d RR: 0.3	VFA	3.5 ± 0.7 g/L	Algapani et al. (2019)	The increase in RR from 0.3 to 1 decreased the VFA production in the first reactor. The NH_4 concentration in leachate and its influence on microbial activities could have been explored to better understand the relation between RR and VFA production

Table 3 (continued)

Feedstock	Inoculum	Reactor types, volume	Operating conditions	Targeted organic acids	Total Organic Acid/ VFA Production	Reference	Limitation
FW	Effluent from thermophilic AD plant treating maize straw and mesophilic AD plant treating sewage sludge	CSTR, 2 L	pH: 5.5 Temp. (°C): 55 HRT: 5 days OLR: 16.3 kg VS/ m^3/d; no recirculation	VFA	9.6 ± 1.5 g/L	Algapani et al. (2019)	

[a]Acetic acid
[b]Propionic acid
[c]Butyric Acid
IS: Inoculum to substrate ratio
C:N: Carbon to nitrogen ratio

H_2 during a continuous fermentation process. The most suitable option would be to vent out the H_2 produced at regular intervals or continuously.

Hydraulic retention time (HRT)

In bioreactor systems without sludge recirculation, HRT can refer to the time length that the biomass remains in the bioreactor (Strazzera et al. 2018) which depends upon the volume of the reactor and the flow rate of the feed. Therefore, it is also associated with capital cost. The optimum HRT for the first reactor is 1–3 days whereas for the second reactor is 10–15 days (O-Thong et al. 2018). This is because the H_2 producing bacteria have a faster growth rate compared to methanogenic bacteria.

Similarly, the longer HRT for the second reactor allows enough time for substrate degradation. The highest methane production rate of 2041.7 L CH_4/m^3/day from an anaerobic fixed bed reactor (AFBR) was possible even at the shortest HRT of 1.5 days given the maximum organic loading rate (OLR) of 6.1 kg COD/m^3.day (Yeshanew et al. 2016). This implies that the use of different biofilm reactor technologies such as AFBR that prevent washing out methanogenic organisms. It helps in the reduction of HRT without compromising the reactors 'performance. Though methanogens such as *Clostridiaceae* are highly reduced at low HRT (4–8 h), other homoacetogens like *Clostridium ljungdahlii*, were difficult to remove due to the granular structure of Upflow Anaerobic Sludge Blanket (UASB) and packed bed reactor (PBR) (Si et al. 2015).

Vo et al. (2019) found out that the optimum H_2 (714 mL/L.d) and CH_4 (254 mL/L.d) were produced at an HRT of 2 days. The study experimented with a single-stage AF reactor which consists of two compartments each for H_2 and CH_4 production. The effect of HRT and OLR along with pH on VFA production from biowaste has been explored. Lim et al. (2008) studied the effect of 3 different HRTs (4, 8, and 12 days) at controlled pH 5.5 and OLR of 5 g TS/ L.d. There was an increase in the VFAs concentration (5.5 g/L at 4 days, 13 g/L at 8 days, 22 g/L at 12 days) with an increase in HRT. At shorter HRTs, acetate was the dominant product while at longer HRT (12 days) propionic acid was higher.

Han and Shin (2002) also studied the effect of HRT (0.25, 0.33, 0.50, and 1 d) on VFA production from FW on Leachate bed reactor (LBR) under controlled temperature (35℃) and pH. At an HRT of 1 day, the maximum VFAs concentration of 202 and 181 mmol/L was determined with rumen and anaerobic bacteria, respectively. However, at 0.25 days of HRT, the least VFAs concentration was detected. According to Strazzera et al. (2018), lower HRT (< 10 days) favors VFA production because the longer HRT facilitates methanogenic microorganisms to convert VFA to CH_4.

Organic loading rate (OLR)

Organic loading rate (OLR) refers to the amount of substrate fed into the reactor in a day per unit of working volume. It is a deterministic parameter for the failure of the system due to bulking and acidifications. For CH_4 production from vegetable waste, the optimum OLR for a CH_4 production rate of 0.26m^3 CH_4/kg VS was 1.4 kg VS/m^3.d, and as the OLR increased VS degradation and biogas yield decreased (Jalil et al. 2019). High OLR reduces the hydrolysis and acidogenesis process due to imbalanced osmotic pressure and increased viscosity that limits mass transfer and metabolic activities (Tang et al. 2016).

However, some experiments have also proved better biohythane production even at high OLR under the condition of low HRT (Cavinato et al. 2011; O-Thong et al. 2018; Yeshanew et al. 2016). A stable H_2 (56.6 mL H_2/g VS) and CH_4 (248 mL CH_4/g VS) production were observed under high OLR (> 10 g VS/ L.d) for a two-phase thermophilic continuous stirred tank reactor (CSTR). But under similar OLR the one-phase thermophilic CSTR failed because of acetate and propionate accumulation in the methane reactor (Luo et al. 2010). Another example from the pilot-scale (50L) two-stage AD process, the gas production from FW increased up to 13,000–15,000 L/d due to an increase in OLR at a low HRT of 3.9 days (Lee and Chung 2010).

Jiang et al. (2013) studied the VFA production from FW at 3 different OLR (5, 11, and 16 g TS/L) and constant temperature (35 °C), pH (6.0), and HRT (5 days). The researcher found that the VFA production increased sharply at the initial days (7–14 days) then remained stable for OLR of 5 and 16 g TS/L. However, at OLR of 16 g TS/L, the VFA production increased by 12 days then decreased sharply. Acetate and Butyrate were the dominant (60%) VFA produced at all OLR. Though there is an insignificant change in VFA fraction at high OLR (Paudel et al. 2016; Cheah et al. 2019), Amha et al. (2019) also recorded an increase in VFA production while increasing OLR. Because increasing OLR is attributed to the diverse microbial community (Srisowmeya et al. 2020).

Nevertheless, the two-stage AD process for FW can operate at high OLR but within controlled HRT and pH (Lim et al. 2008; Srisowmeya et al. 2020). High OLR provides excess carbon for degradation inducing acidic conditions in the reactor in the long-term. Maintaining the pH (5–6.5) for DF and low HRT (2–3 days) that helps to wash-out methanogenic microorganisms provides an optimum condition for biohythane and VFA production at high OLR in a two-stage AD process (Cavinato et al. 2016; Paudel et al. 2016).

Recirculation ratio (RR) of second stage-effluents

The recirculation ratio (RR) refers to the ratio of the returned volume of the second-stage reactor effluent to the volume of the first stage reactor influent. The digestate from the methanogenic stage is recirculated to maintain the pH of the first phase reactor (Algapani et al. 2019). This innovative strategy utilizes the residual buffer capacity of digestate to supply nutrients in the first stage reactor, i.e., DF reactor. This has proved to enhance the biohythane production for two-stage reactors and improves its cost-effectiveness (Micolucci et al. 2014; Cavinato et al. 2011).

In a study conducted by Yeshanew et al. (2016), the supernatant effluent from AFBR was fed to CSTR at a RR ranging from 0.24–0.48, 0.5–0.8, and 0.6–1 for three different periods, respectively. An improved and stable hydrogen production rate of 178.2, 253.5, and 391.7 L H_2/m^3.d in periods I, II, and III, respectively was obtained (Yeshanew et al. 2016). Similarly, Algapani et al. (Algapani et al. 2019) stated that the RR reduces the need for external chemicals to maintain the pH in the H_2 reactor by 54%. It also increased the H_2 by 8% and decreased the CH_4 production by 3%. However, there was no difference in the total energy production (Algapani et al. 2019).

According to Chinellato et al. (2013), the first stage reactor produced CH_4 at a high OLR of 20 kg TVS/m^3/d instead of H_2. However, with the RR of 2.9, the highest H_2 production from FW was obtained at 20 kg TVS/m^3/day. Similarly, for the VFA production from LBR, improving the leachate recirculation rate improved the hydrolysis performance by 10–16% and the acidification yield increased to 340 g COD/kg TVS added (Hussain et al. 2017).

Although many such experiments have proved to enhance the efficiency of biohythane production (Chu et al. 2008; Chinellato et al. 2013; Cavinato et al. 2016), the increased ammonia concentration in the first reactor could confine the performance of anaerobic microorganisms known for both H_2 and CH_4 production (Cavinato et al. 2012; Algapani et al. 2019). Therefore, it is recommended to daily observe the ammonia concentration and remove the recirculation of the second reactor effluent once the ammonia concentration reaches its maximum stable value (Cavinato et al. 2012).

Based on the literature, the optimum conditions in the two-stage AD process for H_2, VFA, and CH_4 production from the FW are summarized in Table 4. H_2 production from DF was found to be suitable within the acidic range unlike for VFA and CH_4 production. The selection of pH value for VFA also depends upon the targeted organic acid. Similarly, the temperature range is within the mesophilic to

thermophilic conditions. From the limited available studies, a low range of HPP (below atmospheric pressure) is suitable for the DF-AD process. The OLR and RR vary depending upon the HRT and the reactor size.

DF-AD process integration

The coupling of DF and AD as two-staged biorefinery is observed to be more productive in terms of energy recovery not only because of the production of biohydrogen but also because of higher yields of CH_4 compared to an uncoupled system (Ruggeri et al. 2015; Ghimire et al. 2021). This is seen as a result of biological pretreatment offered by DF by breaking the nutrients in the substrate down to VFAs (Malave' et al. 2015; Malavè et al. 2018). The reactor type and configuration strongly influence the coupled system, especially through biomass retention (Show et al. 2011).

It is seen that the DF-AD system is integrated widely in CSTRs connected in series with a batch, continuous or semi-continuous feeding, and effluent extraction mechanism (Table 2). In batch mode, the substrate is added to the digester and inoculated for complete digestion, whereas, in continuous feeding mode, the substrate is fed continuously or semi-continuously, and biogas is collected continuously (Stalin and Prabhu 2007). The CSTR is suitable for FW with a TS of 2–12% (Liu et al. 2013). Moreover, two CSTRs have been combined vertically that produced maximum H_2 and CH_4 content of 8.6% and 48%, respectively, from the FW at HRT of 2 days (Vo et al. 2019). The popularity of CSTR is attributed to its simplicity, uniform mixing, and suitability with any kind of substrate (Saratale et al. 2019). However, at HRTs lower than 2.5 days, CSTR enabled mass cell washout from the reactor (Kongjan and Angelidaki 2010).

For the two-stage process stability and better performance, CSTR has also been combined with high state reactors like AFBR, UASB, Up-flow Anaerobic Packed Bed (UAPB), and Expanded Granular Sludge Bed (EGSB) (Lay et al. 2010; Liu et al. 2013; Cisneros-Pérez et al. 2017). These wet reactors are suitable for liquid substrate ($TS \leq 2\%$) and facilitate biomass retention for a longer time which is appropriate for slow-growing methanogenic organisms; hence, mostly used as the second-stage reactors in the two-stage AD process. (Liu et al. 2013; Nualsri et al. 2016; Van et al. 2020).

A study by Ren et al. (2010) has concluded that attached sludge CSTR is more stable than suspended sludge CSTR. A study comparing CSTR and AFBR in immobilized and suspended cell systems concluded that DF is enhanced in reactors that aid biofilms (Qureshi et al. 2005). The recirculation strategy in these reactors' configurations allows maximum H_2 production from DF even at HRT of less than 2 days (Yeshanew et al. 2016).

A settler tank can also be added in between CSTR and UASB, or similar reactors for solid–liquid separation. Lee and Chung (2010) developed the first pilot-scale system consisting of CSTR (500 L) as DF, UASB (2300 L) as AD fed with FW liquid using anaerobic sludge as the inoculum, and a fuel cell fed with the purified biohydrogen. However, CSTR and UASB have been successfully integrated for biohythane recovery from sugarcane without the need for a separation tank in between (Nualsri et al. 2016).

Another novel two-stage AD process has been an integration of dry reactors like LBR with UASB for biohythane (Han and Shin 2004) and VFA production (Browne et al. 2013; Yan et al. 2019) from FW. The dry reactor is suitable for FW with $TS \geq 15\%$(Liu et al. 2013; Van et al. 2020). Han and Shin (2004) used BIOCELL (LBR-UASB) which has VS conversion efficiency to H_2 was 28.2% and CH_4 69.9%, respectively, from FW. The BIOCELL demonstrated stability through the resource recovery process. LBR has several advantages to CSTR as LBR doesn't require substrate dilution reducing process water, no stirrer for mixing that saves energy and the leachate can be recycled without passing it through the solid–liquid separation unit (Browne et al. 2013; Hussain et al. 2017). Similarly, the bacterial dynamics in LBR can degrade even the resistant dietary fibers present in FW (Xiong et al. 2019).

Various types of membrane filtration reactors (MFR) such as microfiltration, nanofiltration, evaporation, electrodialysis, and ultrafiltration have been used to separate the VFAs (Zacharof and Lovitt 2014; Sasiradee et al. 2017). Short Chain Fatty Acids (SCFA) yield of 7453 mg COD/L was recorded from the integration of alkaline sludge fermenter and membrane separation unit (Longo et al. 2015). The combination of anaerobic membrane filtration technologies is more efficient than the single step in recovering the high quality of targeted organic acid (Sasiradee et al. 2017; Sikder et al. 2012). Besides, the appropriate selection of adsorption media is essential in membrane filtration technology as it affects the removal efficiency (Uslu 2009). Out of the four different ion exchange resins (IRA-900, IRA-400, IRA-96, and IRA-67), Luongo et al. (2019) recorded IRA-67 recovered 97% of LA in batch test whereas the desorption efficiency in fixed bed reactor was only 68%. Similarly, Amha et al. (2019)found ceramic membrane favorable for FW treatment in long-term without irreversible fouling.

The working volume and flow rates of DF and AD systems vary according to the operating mechanism and the speed of reactions. The difference between the equivalent working volumes of the different stages of anaerobic is symbolized by the coupling ratio. Contorted coupling ratio could result in reactor failure or inefficient operation (Srisowmeya et al. 2020). From Table 2, it can be observed that the size

of the second reactor is either equal to or greater than that of the first reactor in all the cases. As DF has the potential to process higher OLR than AD, the reactor size for AD is correspondingly seen to be higher than that of DF (Ren et al. 2011).

The DF-AD reactor configurations for FW experimented at laboratory or pilot scale have been shown in Fig. 3. The second-stage reactor configuration depends upon the characteristics of effluent from the first stage reactor (Liu et al. 2013). Similarly, the performance of each reactor along with its drawback is summarized in Table 5.

Overall, the selection of DF-AD reactor configuration depends upon the TS content of the feedstock, targeted VFA, and process stability required. In addition to a combination of two-stage reactors, an additional unit like aerobic denitrification tank (Lee et al. 2010) and zeolite adsorption unit (Petracchini et al. 2018) for further treatment of leachate before recycling to the first reactor. This is done to avoid the inhibition by ammonia in the first reactor and ensure long-term stability.

Pretreatment of FW and inoculum

FW composition is heterogeneous which may cause technical instability in the two-stage AD process (Cesaro and Belgiorno, 2014), especially during the start-up phase. The carbohydrate-rich FW is favorable for DF whereas, protein and lipid are difficult to degrade. To catalyze the decomposition process in DF, the inoculum is added. The inoculum obtained from the sludge of other waste treatment plants and breweries contains mixed culture (Alibardi and Cossu 2015; Cappai et al. 2018; Algapani et al. 2019). The mixed culture induces ecological interactions and competition between various H_2 consuming and H_2 producing microorganisms for the same organic substrate which ultimately reduces the performance of the reactor. The H_2 producing microorganisms have a better chance to survive the harsh conditions during the pretreatment of inoculum than the non-spore-forming bacteria such as methanogens and can germinate when favorable conditions are provided to them (Li and Fang 2007).

The pretreatment of FW is usually done to improve the hydrolysis of hardly biodegradable compounds, while inoculum pretreatment is done to enrich H_2 producing microorganisms and inactivate the consumers like methanogens during the start-up of the DF process (Wang and Yin 2017) and improve H_2-CH_4 yield in two-stage AD process (Lee and Chung 2010; Yin et al. 2014). The inoculum pretreatment may be only necessary for the start-up phase while the FW pretreatment is a continuous process. The common pretreatment measures adopted by the researchers for the FW and the enrichment of H_2 producers are explained in the following sections.

Heat treatment

Heat treatment of inoculum for the enrichment appears to be common, simple, inexpensive, and effective (Li and Fang 2007; Wang and Wan 2008). During the heat-shock treatments, the inoculum is heated at 100° C for 30 min under atmospheric pressure (Jariyaboon et al. 2015; Akhlaghi et al. 2019; Sun et al. 2019). The acidogenic bacteria can form spores and ensure their survival whereas methanogens are deactivated in those conditions (Li and Fang 2007). The results after heat treatment did not show any traces of CH_4 in the gas composition obtained after DF. Wang and Wan (2008) found out that heat-shock treated digested sludge increased the substrate degradation efficiency by 97.2%. It further improved H_2 yield (221.5 mL/g glucose) and biomass concentration (2739 mg/L). In DF, Ghimire et al. (2016) reported that H_2 yield from heat-shock treated activated sludge increased by two folds than the anaerobic sludge.

The substrates can be pretreated to enhance the conversion of substrates to intended products. During the heat pretreatment such as steam explosion, the substrate is heated under high temperature (160–260 °C) and pressure (7–50 bar) for a short duration (30 s–20 min) (Keskin et al. 2019). Then, the pressure is quickly released which causes an explosive effect. This pretreatment is suitable for substrates with high lignocellulose (Eg: wheat straw, corn starch) that is commonly found in food industry waste. The explosion improves its digestibility by 90% than steam pretreatment without explosion (Pielhop et al. 2016). This method has been commonly used in the solubilization of sewage sludge (Donoso-Bravo et al. 2015; Dereix et al. 2006) but can be also utilized for the FW pretreatment. However, the steam

Table 4 Optimum range of operational parameters for H_2, VFA, and CH_4 production from FW using DF-AD process

Reactor	Products	Operational parameters					
		pH	Temp (℃)	HRT (days)	HPP (bar)	OLR (kg VS/m^3/d)	RR
DF (first stage)	H_2	5–6.5	33–55	1.3–5	0.11–1	16–18	0.3–2.9
	VFA	5–8	30–50	3–12	0–0.104	-	0.3–1
AD (second stage)	CH_4	5–7.8	35–55	5–15		4–6	-

explosion pretreatment of FW makes it more dilute decreasing the acetic acid and lactic acid concentration (Svensson et al. 2018).

Another similar heat pretreatment method under high temperature and pressure is known as hydrothermal pretreatment. In this method, at high temperature and pressure, the ionized products of water are increased which hydrolyze the macromolecules resulting in a noticeable improvement in FW fermentation (Yin et al. 2014). The suitable temperature was reported to be 160 °C, which gave a VFA yield of 0.908 g/g $VS_{removal}$, 47.6% higher than the control. However, higher temperatures can result in the production of toxic compounds that inhibit microbial activities. Similarly, Ding et al. (2017) recorded 140 °C as the optimum temperature for hydrothermal heat pretreatment of FW as it favored efficient solubilization of carbohydrates and protein. The H_2 and CH_4 yield from two-stage AD was recorded to be 43.0 mL/g VS and 511.6 mL/g VS. The energy conversion efficiency of pretreated FW increased by 31.7% than the untreated FW.

Chemical treatment

Methanogens are strict anaerobes and are very sensitive to many chemicals. It has been found that CH_4 production drops sharply at a pH of below 6.3 or above 7.8 (Li and Fang 2007). The chemical treatment involves the introduction of acidic/alkali compounds to maintain the desired pH of the reactor. Acid pretreatment is efficient in solubilizing carbohydrates whereas alkali treatment is suitable for solubilizing protein, lipids, and lignin (Parthiba Karthikeyan et al. 2018).

Jang et al. (2015) also recorded the highest H_2 production by alkali-shock treatment of FW with 6 N KOH at pH 11 and 12 and without using any co-substrate. Vavouraki et al. (2013) also recorded that pretreatment of a kitchen waste under 1.12% HCl for 94 min or 1.17% HCl for 86 min (at 100 °C) increased the soluble sugar concentration by 120% compared to the untreated kitchen waste. According to Lamaison et al. (2015), acid treatment of the anaerobically digested sludge from sugarcane vinasse treatment plant is better than the heat-treated sludge in long-term. Because the acid pretreated sludge favored the growth of *Clostridiaceae* microbes which consumes the lactate concentration that is known for H_2 inhibition. Likewise, the alkaline pretreatment of raw rice straw using 4% and 8% NaOH at 55 °C for 24 h, increased the H_2 yield by 26 and 57-fold, respectively (Ghimire et al. 2016).

The chemicals like sodium 2-bromoethasulfonic acid (BESA), iodopropane, chloroform, and acetylene are commonly used as methanogenic chemical inhibitors (Wang et al. 2011; Li and Fang 2007; Zhu and Beland 2006; Venkata et al. 2008; O-Thong et al. 2009). BESA inhibits the activity of the co-enzyme M reductase complex which is a chief component for methanogenesis (Venkata et al. 2008; Zhu and Beland 2006). According to Zhu and Beland (2006), BESA is the most cost-effective pretreatment method for inhibiting methanogens without impacting the H_2 producing microorganism in digested sludge.

Another chemical pretreatment with ozone is quite popular for waste-activated sludge (Carrère et al. 2010) but it is least explored with FW. Ariunbaatar et al. (2014) obtained a negligible increase in CH_4 production from the ozone-treated FW. This could be mainly because a high amount of fermentable sugar lost at a high ozone dose.

The chemical pretreatment has been combined with heat and ultrasonic treatment for the effective conversion of FW to H_2 and CH_4. Monlau et al. (2012) compared seven different types of thermo-chemical pretreatment of sunflower stalk. Chemicals (NaOH, H_2O_2, Ca $(OH)_2$, HCl, and $FeCl_3$) were used to vary the pH from 2.3 to 12.2, and the temperature was maintained at 55 °C and 170 °C. Alkaline pretreatment (4% NaOH) at 55℃ for 24 h is recommended for the lignocellulose substrate (Monlau et al. 2012). Similarly, Elbeshbishy et al. (2011) found that the combination of ultrasonic sound and acid produced the highest H_2 yield of 118 mL/g $VS_{initial}$ and VFA concentration 16,900 mg COD/L.

The addition of chemicals is found to be quite an expensive pretreatment method and hence, least preferred. Moreover, concentrated acid pretreatment is required for the efficient hydrolysis of biomass with high cellulose content. As acids are highly corrosive, specific materials are needed to construct the reactor. This adds up to the capital cost making it an expensive option (Keskin et al. 2019). Similarly, the FW or inoculum needs to be neutralized after the chemical treatment which makes it complicated (Banu et al. 2020).

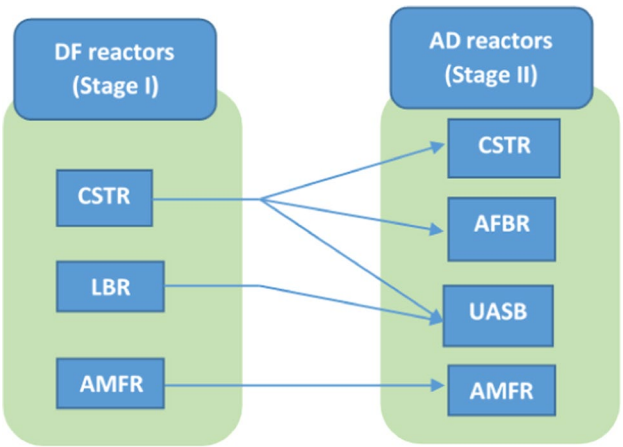

Fig. 3 Available DF-AD reactor configuration for FW in literatures

Table 5 Performance, microbial characteristic, and drawbacks of DF-AD bioreactors

Reactors	Performance	Microbial Characteristics	Drawbacks
CSTR-Batch, semi-continuous, continuous	Lab-scale (500 mL) to pilot-scale (500 L)	Microbes are suspended in CSTR which allows the better mass transfer. But, CSTR cannot retain a greater quantity of fermenting microbes due to rigorous mixing	Substrate dilution required
	Suitable for the substrate with TS:2–12%		Cell wash-out could occur at short HRTs (< 1.5 days)
	Continuous agitation of substrate and inoculum providing uniform composition throughout the reactor		
Leachate Bed Reactor (LBR)	Pilot-scale of LBR up to 10 L for FW	The microbial community in LBR depends upon the pH. At pH 6, bacteria responsible for the hydrolysis of carbohydrates were prominent whereas, at pH, the microbial community is diverse that degrades resistant dietary fibers	Less homogeneity of FW distributed in LBR
	Suitable for the substrate with TS > 15%		
	In LBR, the top section is loaded with FW and the bottom section collects the leachate		Clogging is more likely to occur
	The collected leachate is recirculated to the top reactor by spraying which allows uniform contact between microorganisms and substrate		
	Doesn't require external process water for dilution and energy for mixing, hence, economical		
Anaerobic fluidized bed reactor (AFBR)	Suitable for the substrate with TS < 12%	AFBR facilitates the natural formation of biofilm consisting of complex microbial communities which retain themselves even in fluctuating environmental conditions	
	Favorable even in low HRT, high OLR		Requires mixing
	Effluent from AFBR, when recirculated to the first reactor, provides alkalinity and pH control		Energy intensive and expensive
	Bed fluidization improves homogenous mixing and mass transfer		
Up-flow anaerobic sludge bed reactor (UASB)	Pilot-scale up to 2300L	The microbial community develops on the surface of tiny granules of sludge which further agglomerates to form thickened biofilm called granules. These granules are capable to adapt even to sudden changes in operating parameters that can arise in continuous operations	Energy intensive and expensive
	Stable CH_4 and H_2 production in reduced HRT		

Table 5 (continued)

Reactors	Performance	Microbial Characteristics	Drawbacks
Anaerobic membrane filtration reactor (AMFR)	Reduces VFA and ammonia inhibition in the first reactor	At low HRT, the microbial macromolecules and untreated substrate increase. This forms a second layer that can contribute to high organic removal efficiency	Requires high up-flow velocities
	Provides sufficient buffering capacity		
	Combination of anaerobic and membrane processes used for purification of H_2 and CH_4 or separation of targeted VFA		The performance of MFR fluctuates under low HRT and membrane fouling may occur
	The type of membrane and microbial population affects biohythane and organic acids productivity		Energy intensive

Other pretreatment methods

In the AD process, oxygen is avoided as it inhibits the activity of anaerobic fermentation microorganisms. However, aeration pretreatment of FW in single-stage AD process has been proven to enhance microbial diversity leading to improved hydrolysis and acidogenesis process (Lim and Wang 2013; Girotto et al. 2016). Even in a two-stage process, pre-aeration of FW has increased VFA production (Xu et al. 2014). According to Rafieenia et al. (2017), the pre-aerated FW produced less H_2 than the non-aerated FW. But the CH_4 production increased in the second reactor, especially the protein-rich FW produced the highest CH_4 (351 ml/gV). The effect of aeration pretreatment depends upon the FW composition, aeration time, intensity, and air introduction method (Girotto et al. 2016; Rafieenia et al. 2017).

Moreover, researchers have also studied the effect of aeration pretreatment of inoculum on H_2 production (Wang and Wan 2008; Zhu and Beland 2006). The comparison of the results of pretreatment methods (i.e., acid, base treatment, heat shock, aeration, and chloroform) showed that the inoculum pretreated with aeration has higher cumulative H_2 production than the inoculum treated with chloroform and untreated inoculum (Wang and Wan 2008).

Load shock as a pretreatment was also carried out to obtain high glucose loading of 50 g/L at the beginning of fermentation (Jariyaboon et al. 2015). Different pretreatment methods of inocula for optimizing hydrogen production conducted by (Chang et al. 2011) consisted of acid, base, heat-shock, aeration, chloroform, and 2-bromoethanesulfonate treatment. Acid pretreatment at pH 3 yielded the best result with maximum hydrogen production of 22.81 mmolH_2/gVSS from activated waste sludge.

Cisneros-Pérez et al. (2017) compared a cell wash-out treatment and heat pretreatment of the inoculum in an anaerobic fluid bed reactor (AFBR). This study showed that cell wash-out treatment had higher H_2 volumetric production rates (7 L H_2/L-d) and yields than thermal treatment (3.5 mol H_2/mol hexose). The effect of pretreatment on H_2 production is different based on different sources of inoculum. However, the selection of the pretreatment method should be based on the use of effluent from dark fermentation. For example, the selection of chemical treatment methods such as using BESA could create a problem when the effluents from dark fermentation are meant to be used for methane production in the second stage.

For FW with a high TS loading rate, ultrasonic pretreatment could be a good option (Gadhe et al. 2014). Ultrasonic pretreatment mechanically disrupts the physical, biological, and chemical properties of a substrate/ inoculum under varying frequency (Pilli et al. 2011). Gadhe et al. (2014) recorded 75% enhanced H_2 production by ultrasonically

treating the FW of TS content72,500 mg/L at 1200 W for 5, 10, and 15 min depending upon the specific energy input. Elbeshbishy and Nakhla (2011) also recorded increased efficiency in FW degradation along with an increase in H_2 and CH_4 production rates. But, ultrasonic pretreatment is energy-intensive and expensive hence, requires optimization of operational parameters to set up a full-scale plant (Pilli et al. 2011).

Moreover, commercial enzymes (glucoamylase, proteases, viscozyme, and lipases) have also been used to enhance hydrolysis (Moon and Song 2011; Uçkun Kiran et al. 2015; Donoso-Bravo et al. 2015). According to a study, a mixture of enzymes resulted in higher VFA production than the use of a single enzyme (Kim et al. 2006a). But, the cost of treating 1 ton of FW using glucoamylase is around USD 120 which is expensive (Uçkun Kiran et al. 2015). Therefore, microorganisms such as fungal biomass that is rich in glucoamylase and protease have been used for the efficient production of biomethane from FW (Uçkun Kiran et al. 2015).

Several researchers have reviewed and compared various pretreatment technologies for substrate and inoculum within the biorefinery framework (Galbe and Wallberg 2019; Bhatia et al. 2020; Karthikeyan et al. 2018; Banu et al. 2020). Although thermal pretreatment of FW has been commonly practiced because of its simplicity and cost-effectiveness, not a single pretreatment method could be termed as the "best"(Galbe and Wallberg 2019). The choice of pretreatment technologies depends upon the desired end product and the FW composition (Banu et al. 2020). Some of the researchers have also integrated more than two pretreatment methods (such as acid-thermal) to optimize the performance of the two-stage AD process.

Utilization of VFAs for polyhydroxyalkanoates (PHAs), polylactate, and microalgal biomass production.

With the increasing concern for sustainability and the environment, bio-plastics are becoming popular. By 2025, global demand for bio-based plastics will increase to 2.87 million tonnes from 2.11 million tonnes in 2020 (European Bioplastics 2018). One of the precursor chemicals used for biodegradable plastic production is polyhydroxyalkanoates (PHAs). PHAs are a type of bio-polyesters accumulated by different bacterial cells in the form of granules inside the cytoplasm. The unique thermal and mechanical behavior makes it a completely biodegradable and suitable alternative to plastics (Reis et al. 2011). Producing PHAs from VFA under low temperature, HRT conditions to prevent methanogenic activity has been known to be an affordable process (Beccari et al. 2009). Besides, using mixed microbial culture and VFA rich substrates like FW from AF and sewage sludge reduces the PHA production cost while optimizing PHA yield at the industrial level (Van Aarle et al. 2015; Serafim et al. 2004). Perez-Zabaleta et al. (2021) obtained the highest PHA concentration (3.3 g/L) and content (43.5% w/w) from VFA effluent rich in caprionic and acetic acid, respectively.

Within VFA, butyrate was recorded to produce 57% of polyhydroxybutyrate (PHB) which is higher than with acetate at 54% in sequence batch reactor (SBR), making it the desirable substrate for PHBs (Marang et al. 2013). The production of PHA from organic acid derived from the OFMSW has also been studied for a pilot-scale anaerobic percolation bio cell reactor (100 L). The study showed a PHA production of 223 ± 28 g/kg with a hydroxybutyrate/hydroxyvalerate (%) ratio of 53/47 under optimized acidogenic fermentation (Colombo et al. 2017). Though FW is a potential source to produce PHA, it could be an expensive method considering the pretreatment of FW, transportation cost, and microbial strain selection (Nielsen et al. 2017).

The DF effluents containing VFA can be also used for the production of microalgal biomass (Fei et al. 2015; Turon et al. 2015; Ren et al. 2014). Microalgae are unicellular microorganisms that can be a rich source of carbon compounds. The use of microalgae is increasing especially in the industrial sector as biofuels, by-products, health supplements, pharmaceuticals, and cosmetics (Chalima et al. 2017). They have also been applied for wastewater treatment and CO_2 sequestration (Krishna et al. 2012). According to Lacroux et al. (2020), the growth of a mixed group of microalgae depends upon the concentration of VFA determined in the ranges of 71–207 mg/L and 13–25 mg/L as threshold concentrations for microalgae growth in acetic acid and butyric acid, respectively.

VFA is a byproduct derived from the fermentation of FW and thus, is known to be a cost-effective carbon source. This could be a possible alternative to expensive refined glucose for large-scale microalgal production. Fei et al. (2011) carried out a preliminary cost analysis on using VFA for cultivating *C. albidus* for biodiesel production. The study showed that VFAs-based biodiesel production was affordable compared to the use of agricultural products for lipid accumulation. In another study, Fei et al. (2015) used VFA as a carbon source to yield 0.187 g/g lipid coefficient and 48.7% lipid content of *Chlorella protothecoides*.

Moreover, the microalgae can directly convert the VFA into acetyl-CoA by acetyl coenzyme-A synthetase which could be used for fatty acid biosynthesis and lipid accumulation. Therefore, the use of VFA for microbial mass production could be favorable in terms of time and money. However, limited researches are available on the use of VFA derived from organic waste for microbial cell growth and especially lipid production. Most of the researches has focused on VFA production from microalgae. Therefore, there is a huge scope for further process development on using VFA for microbial-derived protein production for animal feed and energy biomass.

Economic prospects of biohythane and biochemicals

Besides the optimization of the two-stage AD process, some studies covered the economic viability for the production of biohythane and biochemical (Urbaniec and Grabarczyk 2014; Han, et al. 2016a, b; Bastidas-Oyanedel and Schmidt 2018). Bastidas-Oyanedel and Schmidt (2018) compared the economic advantage of combining DF with LA fermentation technologies for the conversion of 50 tonnes/day of FW into CH_4, power generation, LA, PLA, H_2, acetic acid, and butyric acid. Besides the power generation, all other alternatives were profitable. The highest profit of 296 $/tonne VS was obtained from DF with the separation and purification of butyric acid.

Similarly, the novel biohythane process (pretreatment, DF with *Caldicellulosiruptor saccharolyticus* and AD) resulted in high H_2 productivity with 6.1 L/L/d of pure biohythane and 69% of energy recovery from the sugar fraction of the wheat straw. But, the sparging of DF has high energy demand leading to the increased operational cost. Moreover, the addition of nutrients for high H_2 productivity also added to the total production cost of 160 Euro/GJ biofuel (Willquist et al. 2012). A two-stage AD of sugarcane (vinnase) showed 20–30% better economic performance than single-stage AD (Fuess et al. 2018). The net present value (NPV) was found to be $208.58–219.86 million. Besides, optimizing the alkalization of methanogenic systems improves both the economic and environmental performance of AD plants by reducing operating costs and the risks associated with human toxicity and freshwater eutrophication (Fuess et al. 2018).

Another large scale (50 m^3) DF was found to be economically feasible as its return on investment was higher than

Table 7 Bulk prices and market size of major DF products (Adapted and modified from (Bastidas-Oyanedel et al. 2019))

Compound	Price (USD/tonne)	Market size (tonne/year)
Acetic acid	400-800[a,b]	3,500,000[a]
Butyric acid	2000-2500[a,b]	30,000[a]
Propionic acid	1500-1700[a,b]	180,000[a]
Caproic acid	2000-2500[a,b]	25,000[a]
Lactic acid	1000-2100[a,b]	120,000[a]
Formic acid	950–1200[a,b]	30,000[a]
Hydrogen	600-1800[c]	-
Ethanol	800-1500[d]	87,931,200[e]
Derivative products		
Polyhydroxyalkanoates (PHA)	1000 – 5200[d]	29,540[f]
Polylactic acid (PLA)	2000 – 4500[d]	217,330[f]
FAME biodiesel	500 – 850[d]	29,920,000[e]

[a]Zacharof and Lovitt (Zacharof and Lovitt 2013)

[b]Alibaba as cited in (Bastidas-Oyanedel et al. 2019)

[c]Bastidas-Oyandel and Schmidt (Bastidas-Oyanedel and Schmidt 2018)

[e]Alibaba (Alibaba 2020)

[e]REN21 (REN21 2019)

[f]IEA Bioenergy (IEA Bioenergy 2020)

Table 6 H_2 production cost within food industry based on different processes and pretreatment

Industry	Feedstock	Pretreatment	Processes	H_2 production cost	References
Food	Barley straw	Acid-catalyzed steam	DF + PF	51.0 Euro/kg	Ljunggren et al. (2011)
Food	Food waste	Enzymatic	SSF + DF	25.45 USD / kg	Han, et al. (2016a, b)
Food	Wheat straw	Steam explosion	DF + AD	160 Euro/GJ biofuel	Willquist et al. (2012)
Food	Food waste	-	DF + AD	60 USD/t_VS	Bastidas-Oyanedel and Schmidt (2018)
Food	Waste bread	-	CSTR	14.89 USD /kg H2	Han, et al. (2016a, b)
Food	Sugar beet molasses	-	DF + PF	31.8 Euro/kg (base) 31.92 Euro/kg (update) 9.30 Euro/kg (optimistic)	Urbaniec and Grabarczyk (2014)

the DF of 10 m^3. The net present value, payback period, and internal rate of return with a scale of 50 m^3 were USD 526,551, 6.9 years, and 9.25%, respectively. However, the major drawback of DF was the low hydrolysis rate and nutrient conversion efficiency (Han, et al. 2016a, b). Besides AD, DF has been integrated with solid-state fermentation (SSF) reactor to produce H_2 from FW at a large scale. Considering the waste from the food industry, the H_2 production cost is comparatively low (Table 6). The unit cost of an H_2 production plant with a capacity of 10 tonnes/day and a lifetime of 10 years was USD 2.29/m^3 (25.45 USD/kg) which was less than the market price of USD 2.7/m^3 (30.0 USD/kg) (Han, et al. 2016a, b).

Similarly, Urbaniec and Grabarczyk (2014) compared the H_2 production cost under three different scenarios: base, update, and optimistic whose values are based on knowledge, research, and considering two-stage will be fully developed in future, respectively. The H_2 production cost (9.3 Euro/kg) was found lowest in the optimistic case.

According to Ljunggren and Zacchi (2010), the main contributors to the H_2 production cost are the capital costs and the nutrients added. However, with cheap FW as substrate and effluent recirculation in two-stage AD can remove these hurdles. Recently, Li et al. (2020) demonstrated that a large-scale two-stage AD process that treats 216 tons of FW daily is technically and economically feasible. The research team estimated the total investment cost of large scale to be 24.34 million Yuan (1 Yuan is equivalent to 0.15 USD). And, with the selling price of biogas as 0.46 USD/m^3, the total investment can be paid back in 1.5 years. Similarly, the advantage of the biorefinery system converting waste to multiple products (energy, biochemical, & nutrients) and its environmental benefit, trade-off the cost in long run (Krishnan et al. 2019). Nevertheless, more research on the techno-economic study of the two-stage conversion of FW has to be realized in future.

Currently, the industrial production of VFAs is mainly conducted using chemical processes like the oxidation or carboxylation of precursors such as aldehyde and alkenes. However, the continual use of energy-intensive processes contributes to CO_2 emissions and ecological imbalances (Sivagurunathan et al. 2018). The feasibility study of DF conducted in Abu Dhabi showed DF as a promising biorefinery technology not only for the treatment of OFMSW but also for VFA production. The VFA is a versatile chemical as it can be further developed into biofuel or bioplastic in the downstream process. Given a waste tipping fee of 22 USD/tonnes of OFMSW, VFA could be used in downstream processes without any charge. This could make advancement and investment in technology a viable option. The maximum cost of VFA production from DF was calculated to be 15 USD/m^3 effluent (Bonk et al. 2015).

The market size of organic acids in 2013 was reported to be 2.9 million tonnes with a market size of 3.5 billion USD. The market annual growth rate (AAGR) until 2020 is 8.8% (Sun et al. 2019). Table 7 shows the bulk market price and size of the direct DF products and value-added products that can be produced utilizing the DF effluents. Likewise, the bio-polymers (polylactic acid/PLA, PHA) are showing moderate market growth of 9–30% per annum with good future demand for the year 2023 (Sun et al. 2019).

The microbial pathway for biochemical production also influences the cost and yield (Manandhar and Shah 2020; Francois et al. 2020). A study for large-scale (10,000 metric tons per annum) production of lactic acid (LA) from corn determined the production cost of $1181, $1251, and $844 through bacteria, fungi, and yeast pathways were, respectively. The lowest production cost from yeast was due to the lower requirement of chemicals, equipment, and utilities for neutralization and recovery of LA. Moreover, the influencing factors for LA production cost are sugar-to-lactic-acid conversion rates, grain price, plant size, annual operation hours, and gypsum use (Manandhar and Shah 2020). Similarly, the chemical structure of feedstock is another factor affecting the production cost. Based on the study by Daful and Görgens (2017), the cellulose fraction of sugarcane bagasse and brown leaves (SCBL) is economically and environmentally more viable than the hemicellulose fraction of SCBL.

Although FW is a potential source to produce organic acids and biohydrogen, it could be an expensive method considering the pretreatment of food waste, transportation cost, and microbial strain selection (Nielsen et al. 2017; Yun et al. 2018). However, Monlau et al. (2015)suggested that alkaline pretreatment could be economically sustainable if it is applied at a high substrate concentration and/or maximum heat is recovered during pretreatment. Similarly, Colombo et al. (2017) recommended that optimizing the acidic fermentation process (Eg: recirculating the digestate) could support the organic acid formation from OFMSW at a large scale. Also, DF consumes less energy than other conventional technologies for H_2 and VFA production (Bonk et al. 2015). Therefore, the two-stage AD biorefinery process from FW can be a sustainable route to achieving

economic benefits with the least environmental impact (Dahiya et al. 2018).

Conclusion

Biorefinery framework considering two-stage AD process is a promising technology especially DF for H_2 production along with biochemicals that have economic values. The selection of operational parameters depends upon the targeted VFA. Acetate and butyrate are the most abundant VFA produced from FW. Other parameters, OLR and RR also affect the stability of the reactor, biohythane and biochemical production. Generally, OLR and HRT are relative which means low HRT and high OLR enhance the biohythane and VFA production. To enhance the performance of two-stage AD, FW can be pretreated. However, the choice of pretreatment methods and optimum operational conditions depend upon the desired product. The DF-AD process integration also depends upon the TS content of the substrate, type of bioreactors, and coupling ratio.

Despite the high capital investment in two separate bioreactors systems, the DF-AD process can be economically viable if multiple fuels and biochemicals are recovered. Moreover, the market size for VFA production is expanding with an 8.8% growth rate. The two-step process can be a sustainable route to produce biohythane and biochemicals such as butyrate and PHA that have high economic values trades off the cost. Besides the use of biohythane in the industrial sector, the VFA can also be used to produce bioplastic and cultivate microalgae for further energy recovery. Furthermore, various bioreactors configuration within the DF-AD process for FW that can withstand the fluctuation in operational parameters for a longer-term needs to be explored. Similarly, a techno-economic analysis of biohythane and biochemicals recovery from the different FW compositions while optimizing the two-stage AD process needs to be conducted. This would help to determine the viability of a two-stage AD process with FW as a feedstock at a commercial scale.

Authors' contribution SD: conceptualization, Formal analysis, writing original draft; AG: conceptualization, formal analysis, supervision, funding acquisition, contribution in writing the original draft, writing-reviewing, and editing; BT: formal analysis, project administration, supervision, funding acquisition, and visualization; LS: project administration, supervision, and funding acquisition; ST: resources compilation, writing-reviewing; AK: writing-reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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